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(54) Title: GROWTH HORMONE SECRETAGOGUE RECEPTOR FAMILY

(57) Abstract

Human, swine and rat growth hormone secretagogue receptors have been isolated, cloned and sequenced. Growth hormone secretagogue receptors are new members of the G-protein family of receptors. The growth hormone secretagogue receptors may be used to screen and identify compounds which bind to the growth hormone secretagogue receptor. Such compounds may be used in the treatment of conditions which occur when there is a shortage of growth hormone, such as observed in growth hormone deficient children, elderly patients with musculoskeletal impairment and recovering from hip fracture and osteoporosis.

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TITLE OF THE INVENTION

GROWTH HORMONE SECRETAGOGUE RECEPTOR FAMILY

FIELD OF THE INVENTION

This invention relates to a new family of receptors, which includes the growth hormone secretagogue receptors (GHSRs) and growth hormone secretagogue-related receptors (GHSRRs), nucleic acids encoding these receptors; and to the use of a GHSR to identify growth hormone secretagogues and compounds that modulate GHSR function.

BACKGROUND OF THE INVENTION

Growth hormone (GH) is an anabolic hormone capable of promoting linear growth, weight gain and whole body nitrogen retention. Classically, GH is thought to be released primarily from the somatotroph cells of the anterior pituitary under the coordinate regulation of two hypothalamic hormones, growth hormone releasing factor (GHRF or GRF) and somatostatin. Both GHRF stimulation and somatostatin inhibition of the release of GH occurs by the specific engagement of receptors on the cell membrane of the somatotroph.

Recent evidence has been mounting which suggests that GH release is also stimulated by a group of short peptides, the growth hormone releasing peptides (GHRP; GHRP-6, GHRP-2 [hexarelin]) which are described, for example, in U.S. Patent No. 4,411,890, PCT Patent Pub. No. WO 89/07110, PCT Patent Pub. No. WO 89/07111, PCT Patent Pub. No. WO 93/04081, and *J. Endocrinol Invest., 15* (Suppl 4), 45 (1992). These peptides function by selectively binding to distinct somatotroph cell membrane receptor, the growth hormone secretagogue receptor(s) (GHSRs). A medicinal chemical approach has resulted in the design of several classes of orally-active, low molecular weight, non-peptidyl compounds which bind specifically to this receptor and result in the pulsatile release of GH. Such compounds possessing growth hormone secretagogue activity are disclosed, for example, in the following: U.S. Patent No. 3,239,345; U.S. Patent No. 4,036,979; U.S.

Patent No. 4,411,890; U.S. Patent No. 5,206,235; U.S. Patent No. 5,283,241; U.S. Patent No. 5,284,841; U.S. Patent No. 5,310,737; U.S. Patent No. 5,317,017; U.S. Patent No. 5,374,721; U.S. Patent No. 5,430,144; U.S. Patent No. 5,434,261; U.S. Patent No. 5,438,136; U.S. Patent No. 5,494,919; U.S. Patent No. 5,494,920; U.S. Patent No. 5,492,916; EPO Patent Pub. No. 0,144,230; EPO Patent Pub. No. 0,513,974; PCT Patent Pub. No. WO 94/07486; PCT Patent Pub. No. WO 94/08583; PCT Patent Pub. No. WO 94/11012; PCT Patent Pub. No. WO 94/13696; PCT Patent Pub. No. WO 94/19367; PCT Patent Pub. No. WO 95/03289; PCT Patent Pub. No. WO 95/03290; PCT Patent Pub. No. WO 95/09633; PCT Patent Pub. No. WO 95/11029; PCT Patent Pub. No. WO 95/12598; PCT Patent Pub. No. WO 95/13069; PCT Patent Pub. No. WO 95/14666; PCT Patent Pub. No. WO 95/16675; PCT Patent Pub. No. WO 95/16692; PCT Patent Pub. No. WO 95/17422; PCT Patent Pub. No. WO 95/17423; PCT Patent Pub. No. WO 95/34311; PCT Patent Pub. No. WO 96/02530; *Science, 260*, 1640-1643 (June 11, 1993); *Ann. Rep. Med. Chem., 28*, 177-186 (1993); *Bioorg. Med. Chem. Ltrs., 4*(22), 2709-2714 (1994); and *Proc. Natl. Acad. Sci. USA 92*, 7001-7005 (July 1995).

The use of orally-active agents which stimulate the pulsatile release of GH would be a significant advance in the treatment of growth hormone deficiency in children and adults as well as provide substantial benefit under circumstances where the anabolic effects of GH might be exploited clinically (e.g. post-hip fracture rehabilitation, the frail elderly and in post-operative recovery patients).

It would also be desirable to know the molecular structure of growth hormone secretagogue receptors in order to analyze this new receptor family and understand its normal physiological role in concert with the actions of GHRF and somatostatin. This could lead to a better understanding of the *in vivo* processes which occur upon ligand-receptor binding. Further, it would be desirable to use cloned-growth hormone secretagogue receptors as essential components of an assay system which can identify new growth hormone secretagogues.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a novel family of receptors which includes growth hormone secretagogue receptors (GHSRs) and growth hormone secretagogue-related receptors (GHSRRs).

A first aspect of this invention are the growth hormone secretagogue receptors, which are free from receptor associated proteins. GHSRs may be from any species, and in further embodiments may be isolated or purified. One embodiment of this invention is human growth hormone secretagogue receptor (hGHSR), free from receptor-associated proteins. A further aspect of this invention is hGHSR which is isolated or purified.

Another aspect of this invention is swine growth hormone secretagogue receptor (sGHSR), free from receptor-associated proteins. A further aspect of this invention is sGHSR which is isolated or purified.

Another aspect of this invention is rat growth hormone secretagogue receptor (rGHSR), free from receptor-associated proteins. A further aspect of this invention is rGHSR which is isolated or purified.

Another aspect of this invention are human, swine and rat GHSRs which are encoded by substantially the same nucleic acid sequences, but which have undergone changes in splicing or other RNA processing-derived modifications or mutagenesis induced changes, so that the expressed protein has a homologous, but different amino acid sequence from the native forms. These variant forms may have different and/or additional functions in human and animal physiology or *in vitro* in cell based assays.

Another aspect of this invention are the growth hormone secretagogue-related receptors, free from associated receptor proteins. A further embodiment are GHSRRs which are isolated or purified. These may be from any species, including human, mouse, rat and swine.

Growth hormone secretagogue receptors are proteins containing various functional domains, including one or more domains which anchor the receptor in the cell membrane, and at least one ligand binding domain. As with many receptor proteins, it is possible to modify many of the amino acids, particularly those which are not found in the ligand binding domain, and still retain at least a percentage of the biological activity of the original receptor. In accordance with this invention, it has been shown that the N-terminal portions of the GHSR are not essential for its activation by the Growth Hormone Secretagogues (GHSs). Thus this invention specifically includes modified functionally equivalent GHSRs which have deleted, truncated, or mutated N-terminal portions. This invention also specifically includes modified functionally equivalent GHSRs which contain modified and/or deletions in other domains, which are not accompanied by a loss of functional activity.

Additionally, it is possible to modify other functional domains such as those that interact with second messenger effector systems, by altering binding specificity and/or selectivity. Such functionally equivalent mutant receptors are also within the scope of this invention.

A further aspect of this invention are nucleic acids which encode a growth hormone secretagogue receptor or a functional equivalent from swine, human, rat or other species. These nucleic acids may be free from associated nucleic acids, or they may be isolated or purified. For most cloning purposes, cDNA is a preferred nucleic acid, but this invention specifically includes other forms of DNA as well as RNAs which encode a GHSR or a functional equivalent.

Yet another aspect of this invention relates to vectors which comprise nucleic acids encoding a GHSR or a functional equivalent. These vectors may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage and cosmids, yeast artificial chromosomes and other forms of episomal or integrated DNA that can encode a GHSR. It is well within the skill of the ordinary artisan to

determine an appropriate vector for a particular gene transfer or other use.

A further aspect of this invention are host cells which are transformed with a gene which encodes a growth hormone secretagogue receptor or a functional equivalent. The host cell may or may not naturally express a GHSR on the cell membrane. Preferably, once transformed, the host cells are able to express the growth hormone secretagogue receptor or a functional equivalent on the cell membrane. Depending on the host cell, it may be desirable to adapt the DNA so that particular codons are used in order to optimize expression. Such adaptations are known in the art, and these nucleic acids are also included within the scope of this invention. Generally, mammalian cell lines, such as COS, HEK-293, CHO, HeLa, NS/0, CV-1, GC, GH3 or VERO cells are preferred host cells, but other cells and cell lines such as *Xenopus* oocytes or insect cells, may also be used.

Growth hormone secretagogue related receptors are related to GHRS, but are encoded by a distinct gene. The GHRR genes may be identified by hybridization (using relaxed or moderate stringency post-hybridizational washing conditions) of cDNA of GHR DNA to genonic DNA. These sequences have a high degree of similarity to GHR.

Another aspect of this invention is a process for identifying nucleic acids encoding growth hormone secretagogue related receptors comprising hybridizing a first nucleic acid encoding a growth hormone secretagogue receptor with a second nucleic acid suspected of comprising nucleic acids encoding a growth hormone secretagogue, wherein the hybridizing takes place under relaxed or moderate post hybridizational washing conditions; and identify areas of the second nucleic acid where hybridization occurred.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is the DNA of Swine GHSR (type I) contained in Clone 7-3.

FIGURE 2 is the amino acid sequence of swine GHSR encoded by the DNA of Figure 1.

FIGURE 3 is the entire open reading frame of the type I clone of Figure 1.

FIGURE 4 is the DNA of Swine GHSR (type II) contained in Clone 1375.

FIGURE 5 is the amino acid sequence of swine GHSR (type II) encoded by the DNA of Figure 4.

FIGURE 6 is the DNA for human GHSR (Type I) contained in Clone 1146.

FIGURE 7 is the amino acid sequence of human GHSR (type 1) encoded by the DNA of Figure 6.

FIGURE 8 is the entire open reading frame of Type I GHSR, encoded by the DNA sequence of Figure 6.

FIGURE 9 is the DNA for human GHSR (type II) contained in Clone 1141.

FIGURE 10 is the amino acid sequence of human GHSR (Type II) encoded by Clone 1141.

FIGURE 11 is the DNA for human GHSR (Type I) contained in Clone 1143.

FIGURE 12 is the amino acid sequence of human GHSR (Type I) encoded by Clone 1143.

FIGURE 13 compares the ORF of swine Type I (lacking the MET initiator of the full length GHSR and lacking 12 additional amino acids) to the homologous domain of swine Type II receptors.

FIGURE 14 compares the homologous domain of human Type I and Type II receptors (the amino terminal sequence lacks the MET initiator and four additional amino acids).

FIGURE 15 compares the ORFs of swine Type I and human Type I receptors (the amino terminal sequence lacks the MET initiator and 12 additional amino acids).

FIGURE 16 compares full length swine Type II and human Type II receptors.

FIGURE 17 is a schematic diagram depicting the physical map of swine and human growth hormone secretagogue receptor cDNA clones.

FIGURE 18 is a graph demonstrating the pharmacology of the expressed swine and human growth hormone secretagogue receptors in *Xenopus* oocytes using the aequorin bioluminescence assay.

FIGURE 19 is a table demonstrating the pharmacology of the expressed swine and human growth hormone secretagogue receptors in *Xenopus* oocytes using the aequorin bioluminescence assay and various secretagogues.

FIGURE 20 is a graph representing the pharmacology of the pure expressed swine growth hormone secretagogue receptor in COS-7 cells using the ^{35}S-labeled Compound A binding assay.

FIGURE 21 is a table representing the competition analysis with the pure expressed swine growth hormone secretagogue receptor in COS-7 cells using the ^{35}S-labeled Compound A binding assay and various secretagogues and other G-protein coupled-receptors (GPC-Receptors) ligands in a competition assay.

FIGURE 22 is the amino acid sequence of the full length human GHSR (type I) encoded by clone 11304.

FIGURE 23 is the rat GHSR DNA sequence from the Met Initiation codon to the Stop codon. This sequence includes an intron.

FIGURE 24 is the open reading frame only of the rat GHSR of Figure 23.

FIGURE 25 is the deduced amino acid sequence of the ORF of Figure 24.

FIGURE 26 shows the expression of functional rat GHSR in transfected HEK-293 cells.

As used throughout the specification and claims, the following definitions shall apply:

Growth Hormone Secretagogue - any compound or agent that directly or indirectly stimulates or increases the release of growth hormone in an animal.

Ligands-- any molecule which binds to GHSR of this invention. These ligands can have either agonist, partial agonist, partial antagonist or antagonist activity.

Free from receptor-associated proteins-- the receptor protein is not in a mixture or solution with other membrane receptor proteins.

Free from associated nucleic acids-- the nucleic acid is not covalently linked to DNA which it is naturally covalently linked in the organism's chromosome.

Isolated receptor--the protein is not in a mixture or solution with any other proteins.

Isolated nucleic acid-- the nucleic acid is not in a mixture or solution with any other nucleic acid.

Functional equivalent--a receptor which does not have the exact same amino acid sequence of a naturally occurring growth hormone secretagogue receptor, due to alternative splicing, deletions, mutations, or additions, but retains at least 1%, preferably 10%, and more preferably 25% of the biological activity of the naturally occurring receptor. Such derivatives will have a significant homology with a natural GHSR and can be detected by reduced stringency hybridization with a DNA sequence obtained from a GHSR. The nucleic acid encoding a functional equivalent has at least about 50% homology at the nucleotide level to a naturally occurring receptor nucleic acid.

Purified receptor-- the receptor is at least about 95% pure.

Purified nucleic acid-- the nucleic acid is at least about 95% pure.

Compound A -- (N-[1(R)-[(1,2-dihydro-1-methane-sulfonylspiro[3H-indole-3,4'-piperidin]-1'-yl)carbonyl]-2-(phenyl-methyloxy)ethyl]-2-amino-2-methyl propanamide, described in Patchett, 1995 *Proc. Natl. Acad. Sci.* 92:7001-7005.

Compound B -- 3-amino-3-methyl-N-(2,3,4,5-tetra-hydro-2-oxo-1-{[2'-1H-tetrazol-5-yl)(1,1'-biphenyl)-4-yl]methyl}-1H-benzazepin-3(R)yl-butanamide, described in Patchett, 1995 *Proc. Natl. Acad. Sci. 92*:7001-7005.

Compound C -- 3-amino-3-methyl-N-(2,3,4,5-tetrahydro-2-oxo-1-{[2'-1H-tetrazol-5-yl)(1,1'-biphenyl)-4-yl]methyl}-1H-benzazepin-3(S)yl-butanamide, described in U.S. Patent 5,206,235.

Standard or high stringency post hybridizational washing conditions -- 6 X SSC at 55°C

Moderate post hybridizational washing conditions --6 X SSC at 45°C

Relaxed post hybridizational washing conditions -- 6 X SSC at 30°C

The proteins of this invention were found to have structural features which are typical of the 7-transmembrane domain (TM) containing G-protein linked receptor superfamily (GPC-R's or 7-TM receptors). Thus growth hormone secretagogue family of receptors make up new members of the GPC-R family of receptors. The intact GHSRs of this invention were found to have the general features of GPC-R's, including seven transmembrane regions, three intra- and extracellular loops, and the GPC-R protein signature sequence. The TM domains and GPC-R protein signature sequence are noted in the protein sequences of the Type I GHS receptor in Figures 3 and 8. Not all regions are required for functioning, and therefore this invention also comprises functional receptors which lack one or more non-essential domains.

The GHSRs of this invention share some sequence homology with previously cloned GPC-receptors including the rat and human neurotensin receptor (approximately 32% identity) and the rat and human TRH receptor (approximately 30% identity).

The GHSRs of this invention were isolated and characterized using expression cloning techniques in *Xenopus* oocytes.

The cloning was made difficult by three factors. First, prior to this invention, there was very little information available about the biochemical characteristics and intracellular signaling/effector pathways of the proteins. Thus, cloning approaches which depended on the use of protein sequence information for the design of degenerate oligonucleotides to screen cDNA libraries or utilize PCR could not be effectively utilized. In accordance with this invention, therefore, receptor bioactivity needed to be determined.

Secondly, the growth hormone secretagogue receptor does not occur in abundance-- it is present on the cell membrane in about 10 fold less concentration than most other membrane receptors. In order to successfully clone the receptors in accordance with this invention, exhaustive precautions had be taken to ensure that the GHSR was represented in a cDNA library to be screened. This required isolation of intact, undegraded and pure poly $(A)^+$ mRNA, and optimization of cDNA synthesis to maximize the production of full-length molecules. In addition, a library of larger size than normal needed to be screened (approximately 0.5 to 1 x 10^7 clones) to increase the probability that a functional cDNA clone may be obtained.

Thirdly, no permanent cell line which expresses this receptor is known. Therefore, primary pituitary tissue had to be used as a source for mRNA or protein. This posed an additional obstacle because most primary tissues express lower amounts of a given receptor than an immortalized cell line that may be maintained in tissue culture or some tumor materials. Further, the surgical removal of a pig pituitary and extraction of biologically-active intact mRNA for the construction of a cDNA expression library is considerably more difficult than the extraction of mRNA from a tissue culture cell line. Along with the need to obtain fresh tissue continuously, there are problems associated with its intrinsic inter-animal and inter-preparation variability. The development of cell lines expressing a receptor of this invention is therefore a significant aspect of this invention.

Yet another aspect of this invention is the development of an extremely sensitive, robust, reliable and high-throughput screening

assay which could be used to identify portions of a cDNA library containing the receptor. This assay is described and claimed in co-pending patent applications Serial No. 60/008,584, filed December 13, 1995, and Attorney Docket No. 19590PV2 filed herewith.

Briefly, the ability to identify cDNAs which encode growth hormone secretagogue receptors depended upon two discoveries made in accordance with this invention: 1) that growth hormone secretagogue receptor-ligand binding events are transduced through G proteins; and 2) that a particular G protein subunit, G_{a11}, must be present in the cells in order to detect receptor activity. Only when these two discoveries were made could an assay be devised to detect the presence of GHSR-encoding DNA sequences.

When the GHSR is bound by ligand (a growth hormone secretagogue), the G-proteins present in the cell activate phosphatidylinositol-specific phospholipase C (PI-PLC), an enzyme which releases intracellular signaling molecules (diacylglycerol and inositol triphosphate), which in turn start a cascade of biochemical events that promote calcium mobilization. This can be used as the basis of an assay. A detector molecule which can respond to changes in calcium concentrations, such as aequorin, a jellyfish photoprotein, is introduced into a cell along with a complex pool of up to 10,000 individual RNAs from a cDNA expression library, at least one of which may encode a GHSR. The cell is then exposed to a known growth hormone secretagogue, such as Compound A or Compound B. If one or more RNAs encodes a GHSR, then the secretagogue ligand will bind the receptor, G-protein will be activated, the calcium level will fluctuate, and the aequorin will produce measurable bioluminescence. Once a positive result is found, the procedure can be repeated with a sub-division of the RNA pool (for example, approximately 1,000, then approximately 500, then approximately 50, and then pure clones) until a single clone is identified from which RNA can be generated which encodes a GHSR.

Using this general protocol in *Xenopus* oocytes with a swine cDNA expression library, Clone 7-3 was identified as containing

nucleic acid encoding a swine GHSR. The insert of the cDNA clone is approximately 1.5 kb in size, and downstream from the presumed initiator methionine (MET), contains an open reading frame (ORF) encoding 302 amino acids (M_r= 34,516). The DNA and deduced amino acid sequence are given in FIGURES 1 and 2. When hydropathy analysis (e.g. Kyte-Doolittle; Eisenberg, Schwartz, Komaron and Wall) is performed on the protein sequence of clone 7-3, only 6 predicted transmembrane domains are present downstream of the presumed MET initiator. Translation of the longest ORF encoded in clone 7-3 encodes a protein of 353 amino acids (M_r= 39,787); however an apparent MET initiator cannot be identified for this longer reading frame (FIGURE 3). This longer reading frame is significant since 7 transmembrane segments are encoded in the 353 amino acids protein in which a MET translation initiation codon located upstream of TM1 is absent. In addition, this longer protein also shares homology with known G-protein coupled receptors in its predicted TM1 domain (FIGURE 3 and next sections). Thus, clone 7-3 while truncated at its amino terminus, is fully functional, demonstrating that clone 7-3 is but one embodiment of a functional equivalent of a native GHSR.

The resultant cDNA clone (or shorter portions of, for instance only 15 nucleotides long) may be used to probe libraries under hybridization conditions to find other receptors which are similar enough so that the nucleic acids can hybridize, and is particularly useful for screening libraries from other species. Using this procedure, additional human, swine, and rat GHSR cDNAs have been cloned and their nucleotide sequences determined. Further, hybridization of a cDNA to genomic DNA demonstrated that the Type I receptor (see below) is encoded by a single gene that is highly conserved. Human, monkey, rat, mouse, dog, cow, chicken and invertebrate DNA all yielded a single hybridizing species at high stringency post-hybridization conditions. Therefore, this invention is not limited to any particular species.

A swine pituitary library, a human pituitary library, and a rat pituitary library were hybridized with a radiolabeled cDNA derived

from the open reading frame of the swine GHSR clone 7-3. 21 positive human GHSR cDNA clones were isolated and five swine library pools yielded a strong hybridization signal and contained clones with inserts larger than clone 7-3, as judged by their insert size on Southern blots. A single rat cDNA clone was also isolated.

Nucleotide sequence analysis revealed two types of cDNAs for both the human and swine GHSR cDNAs. The first (Type I) encodes a protein represented by clone 7-3, encoding seven transmembrane domains. The full length open reading frame appears to extend 13 amino acids beyond the largest predicted open reading frame of clone 7-3 (353 amino acids). The second (type II) diverges in its nucleotide sequence from the type I cDNA at its 3'-end, just after the predicted second amino acid of the sixth transmembrane domain (TM-6).

In the type II cDNAs, TM-6 is truncated and fused to a short contiguous reading frame of only 24 amino acids, followed by a translation stop codon. Swine clone 1375 is an example of a Type II cDNA (FIGURES 4 and 5). These 24 amino acids beyond TM-6 are highly conserved when compared between human and swine cDNAs. The DNA and amino acid sequences of the human GHSR Type I and II are given in FIGURES 6-12. A full length cDNA encoding the human Type I receptor, that is, a molecule encoding 7-TM domains with an initiator MET in a favorable context preceded by an inframe termination codon is isolated, and termed clone 11304. The predicted ORF of clone 11304 for the full length Type I GHSR measures 366 amino acids (M_r= 41,198; FIGURE 22). The full length human Type II cDNA encodes a polypeptide of 289 amino acids (M_r=32,156; FIGURES 9 and 10).

Sequence alignments performed at both the nucleic acid and protein levels show that Type I and II GHSR's are highly related to each other and across species (FIGURES 13-16). The human and swine GHSR sequences are 93% identical and 98% similar at the amino acid level.

The nucleotide sequence encoding the missing amino terminal extension of swine Type I clone 7-3 is derived from the predicted full length human Type I clone and the human and swine Type II cDNAs. The reading frame of the full length clones extended 13 amino acids beyond the amino terminal sequence of clone 7-3 and this sequence was conserved in 12/13 amino acid residues, when compared between human and swine. The amino terminal extension includes a translation initiator methionine in a favorable context according to Kosak's rule, with the reading frame further upstream being interrupted by a stop codon. A schematic physical map of Type I and II swine and human cDNA clones is given in FIGURE 17.

The rat clone was also further investigated. Sequence analysis revealed the presence of a non-coding intronic sequence at nt 790 corresponding to a splice-donor site (see FIGURES 23, 24, and 25). The G/GT splice-donor site occurs two amino acids after the completion of the predicted transmembrane domain 5 (leucine 263), thus dividing the rGHSR into an amino-terminal segment (containing the extracellular domain, TM-1 through TM-5, and the first two intra- and extra-cellular loops) and a carboxy-terminal segment (containing TM-6, TM-7, the third intra- and extra- cellular loops, and the intra- cellular domain). The point of insertion and flanking DNA sequence are highly conserved, and also present in both human and swine Type I and II cDNAs.

Comparison of the complete open reading frame encoding the rat GHSR protein to human and swine homologs reveals a high degree of sequence identity (rat vs. human, 95.1%; rat vs. swine 93.4%.

The human GHSR can be assigned by fluorescent *in situ* hybridization analysis [FISH; as described in *Cytogenet. Cell Genet* 69: 196 (1995)] to the cytogenetic band 3Q26.2. The mouse gene is located on 3A3.

Human and swine Type I cRNAs expressed in oocytes were functional and responded to Compound A concentrations ranging from 1 mM to as low as 0.1 nM in the aequorin bioluminescence assay. Human or swine Type II-derived cRNAs that are truncated in TM-6

failed to give a response when injected into oocytes and these represent a receptor subtype which may bind the GHS, but cannot effectively activate the intracellular signal transduction pathway. In addition the type II receptor may interact with other proteins and thus reconstitute a functional GHSR. Proteins such as these which may have ligand-binding activity, but are not active in signal transduction are particularly useful for ligand-binding assays. In these cases, one may also over-express a mutant protein on the cell membrane and test the binding abilities of putative labeled ligands. By using a non-signaling mutant which is constitutively in a high affinity state, binding can be measured, but no adverse metabolic consequences would result. Thus non-signaling mutants are an important aspect of this invention.

The pharmacological characterization of human, Type I swine, Type I and rat receptors in the aequorin bioluminescence assay in oocytes is summarized in FIGURES 18, 19, and 26. Peptidyl and non-peptidyl bioactive GHS's were active in a similar rank order of potency as observed for the native pituitary receptor. Independent confirmatory evidence that the Type I GHSR (shown for swine clone 7-3) encodes a fully-functional GHSR is given by the finding that when clone 7-3 is expressed transiently in mammalian COS-7 cells, high affinity (K_D ~ 0.2 nM), saturable (B_{max}~80 fmol/mg protein) and specific binding (> 90 % displaced by 50 nM unlabeled Compound A) is observed for ^{35}S-Compound A (FIGURES 20 and 21).

The GHSR receptors of this invention may be identified by hybridization of a GHSR cDNA to genomic DNA, under relaxed or moderate post hybridizational washing conditions. This analysis yields a discreet number of hybridizing bands. A suitable human genomic library which can be used in this procedure is PAC (as described in *Nature Genetics* 6:84 (1994)) and a suitable mouse genomic library is BAC (as described in *Proc Natl Acad Sci USA* 89: 8794 (1992).

Due to the high degree of homology to GHSRs, the GHSRs of this invention are believed to function similarly to GHSRs and have similar biological activity. They are useful in understanding the biological and physiological pathways involved in an organisms growth.

They may be also used to scan for growth hormone secretagogue agonists and antagonists; as in particular to test the specificity of identified ligands.

Heterotrimeric G proteins, consisting of a, b and g subunits, serve to relay information from cell surface receptors to intracellular effectors, such as phospholipase C and adenylate cyclase. The G-protein alpha subunit is an essential component of the intracellular signal transduction pathway activated by receptor-ligand interaction. In the process of ligand-induced GPCR activation, the Ga subunit of a trimeric Gabg exchanges its bound GDP for GTP and dissociate from the bg heterodimer. The dissociated subunit serves as the active signal transducer, often in concert with the bg complex, thus starting the activation of the intracellular signal transduction pathway. By definition, cell surface receptors which couple intracellularly through G protein interactions are termed GPC-R's. This interaction has mainly been characterized with respect to the type of G-alpha (G_a) subunit which is primarily involved in the signal transduction process. G_a subunits are classified into sub-families based on sequence identity and the main type of effectors to which they are coupled have been characterized: G_s, activate adenylate cyclase; $G_{i/o/t}$, inhibit adenylate cyclase; $G_{q/11}$, activate PI-PLC; and $G_{12/13}$, effector unknown.

Expression of several receptors in heterologous cells has been shown to be increased by the co-expression of certain G_a subunits. This observation formed the basis for the rationale to the use of G_a subunits of several sub-families in conjunction with a source of GHSR (swine poly[A^+] mRNA) to test if a GHS-induced functional response could be measured in the *Xenopus* oocyte system. GHS-induced responses were detected and were found to be strictly dependent on G_{a11} co-expression in this system, an unprecedented finding outlining the specificity of the interaction. Thus another aspect of this invention is a method of detecting a GHS response comprising co-expressing a G_{a11} protein subunit in a cell also expressing a GHSR, exposing the cell to a GHS, and detecting the response.

Ligands detected using assays described herein may be used in the treatment of conditions which occur when there is a shortage of growth hormone, such as observed in growth hormone deficient children, elderly patients with musculoskeletal impairment and recovering from hip fracture, and osteoporosis.

The GHSR and fragments are immunogenic. Thus, another aspect of this invention is antibodies and antibody fragments which can bind to GHSR or a GHSR fragment. These antibodies may be monoclonal antibodies and produced using either hybridoma technology or recombinant methods. They may be used as part of assay systems or to deduce the function of a GHSR present on a cell membrane.

A further aspect of this invention are antisense oligonucleotides nucleotides which can bind to GHSR nucleotides and modulate receptor function or expression.

A further aspect of this invention is a method of increasing the amount of GHSRs on a cell membrane comprising, introducing into the cell a nucleic acid encoding a GHSR, and allowing expression of the GHSR.

A GHS receptor, preferably imobilized on a solid support, may be used diagnostically for the determination of the concentration of growth hormone secretagogues, or metabolites thereof, in physiological fluids, e.g., body fluids, including serum, and tissue extracts, as for example in patients who are undergoing therapy with a growth hormone secretagogue.

The administration of a GHS receptor to a patient may also be employed for purposes of: amplifying the net effect of a growth hormone secretagogue by providing increased downstream signal following administration of the growth hormone secretagogue thereby diminishing the required dosage of growth hormone secretagogue; or diminishing the effect of an overdosage of a growth hormone secretagogue during therapy.

The following, non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Oocyte Preparation and Selection

Xenopus laevis oocytes were isolated and injected using standard methods previously described by Arena, *et al.*, 1991, *Mol. Pharmacol.* 40, 368-374, which is hereby incorporated by reference. Adult female *Xenopus laevis* frogs (purchased from Xenopus One, Ann Arbor, MI) were anesthetized with 0.17% tricaine methanesulfonate and the ovaries were surgically removed and placed in a 60 mm culture dish (Falcon) containing OR-2 medium without calcium (82.5 mM NaCl, 2 mM KCl, 2.5 mM sodium pyruvate, 1 mM $MgCl_2$, 100 m/ml penicillin, 1 mg/ml streptomycin, 5 mM HEPES, pH=7.5; ND-96 from Specialty Media, NJ). Ovarian lobes were broken open, rinsed several times, and oocytes were released from their sacs by collagenase A digestion (Boehringer-Mannheim; 0.2% for 2-3 hours at 18°C) in calcium-free OR-2. When approximately 50% of the follicular layers were removed, Stage V and VI oocytes were selected and placed in ND-86 with calcium (86 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 2.5 mM sodium pyruvate, 0.5 mM theopylline, 0.1 mM gentamycin, 5 mM HEPES [pH=7.5]). For each round of injection, typically 3-5 frogs were pre-tested for their ability to express a control G-protein linked receptor (human gonadotropin-releasing hormone receptor) and show a robust phospholipase C intracellular signaling pathway (incubation with 1% chicken serum which promotes calcium mobilization by activation of phospholipase C). Based on these results, 1-2 frogs were chosen for library pool injection (50 nl of cRNA at a concentration of 25 ng (complex pools) to 0.5 ng (pure clone) per oocyte usually 24 to 48 hours following oocyte isolation.

EXAMPLE 2

mRNA Isolation

Total RNA from swine (50-80 kg, Yorkshire strain) pituitaries (snap-frozen in liquid nitrogen within 1-2 minutes of animal sacrifice) was prepared by a modified phenol:guanidinium thiocyanate

procedure (Chomczynski, *et al.*, 1987 *Anal. Biochem.* 162:156-159, using the TRI-Reagent LS as per the manufacturer's instructions (Molecular Research Center, Cincinnati, OH). Typically, 5 mg of total RNA was obtained from 3.5 g wet weight of pituitary tissue. Poly $(A)^+$ RNA was isolated from total RNA by column chromatography (two passes) on oligo (dT) cellulose (Pharmacia, Piscataway, NJ). The yield of poly $(A)^+$ mRNA from total RNA was usually 0.5%. RNA from other tissues was isolated similarly.

EXAMPLE 3

cDNA Library Construction

First-strand cDNA was synthesized from poly $(A)^+$ mRNA using M-MLV RNAse (-) reverse transcriptase (Superscript, GIBCO-BRL, Gaithersberg, MD) as per the manufacturer's instructions with an oligo (dT)/Not I primer-adapter. Following second-strand cDNA synthesis, double-stranded cDNA was subjected to the following steps: 1) ligation to EcoR I adapters, 2) Not I digestion, and 3) enrichment for large cDNAs and removal of excess adapters by gel filtration chromatography on a Sephacryl S-500 column (Pharmacia). Fractions corresponding to high molecular weight cDNA were ligated to EcoR I/Not I digested pSV-7, a eucaryotic expression vector capable of expressing cloned cDNA in mammalian cells by transfection (driven by SV-40 promoter) and in oocytes using *in vitro* transcripts (initiated from the T7 RNA polymerase promoter). pSV-7 was constructed by replacing the multiple cloning site in pSG-5 (Stratagene, La Jolla, CA; Green, S. *et al.*, 1988 *Nucleic Acids Res.* 16:369), with an expanded multiple cloning site. Ligated vector:cDNA was transformed into *E.coli* strain DH10B (GIBCO-BRL) by electroporation with a transformation efficiency of 1×10^6 pfu/10 ng double-stranded cDNA. The library contained approximately 3×10^6 independent clones with greater than 95% having inserts with an average size approximating 1.65 kb (range 0.8-2.8 kb). Unamplified library stocks were frozen in glycerol at -70°C until needed. Aliquots of the library were amplified once prior

to screening by a modification of a solid-state method (Kriegler, M. in *Gene Transfer and Expression: A Laboratory Manual* Stockton Press, NY 1990). Library stocks were titered on LB plates and then the equivalent of 500-1000 colonies was added to 13 ml of 2 x YT media containing 0.3% agarose and 100 mg/ml carbenicillin in a 14 ml round-bottom polypropylene tube (Falcon). The bacterial suspension was chilled in a wet ice bath for 1 hour to solidify the suspension, and then grown upright at 37°C for 24 hrs. The resultant bacterial colonies were harvested by centrifugation at 2000 x g at RT for 10 min, resuspended in 3 ml 2X YT/ carbenicillin. Aliquots were taken for frozen stocks (5%) and plasmid DNA preparation.

EXAMPLE 4

Plasmid DNA Preparation and cRNA Transcription

Plasmid DNA was purified from pellets of solid-state grown bacteria (1000 pools of 500 independent clones each) using the Wizard Miniprep kit according to the manufacturer's instructions (Promega Biotech, Madison, WI). The yield of plasmid DNA from a 14 ml solid-state amplification was 5-10 mg. In preparation for cRNA synthesis, 4 mg of DNA was digested with Not I, and the subsequent linearized DNA was made protein and RNase-free by proteinase K treatment (10 mg for 1 hour at 37°C), followed by two phenol, two chloroform/isoamyl alcohol extractions, and two ethanol precipitations. The DNA was resuspended in approximately 15 ml of RNase-free water and stored at -70°C until needed. cRNA was synthesized using a kit from Promega Biotech with modifications. Each 50 ml reaction contained: 5 ml of linearized plasmid (approximately 1 mg), 40 mM Tris-HCl (pH=7.5), 6 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 10 mM DTT, 0.05 mg/ml bovine serum albumin, 2 units/ml RNasin, 800 mM each of ATP, CTP and UTP, 200 mM GTP, 800 mM m7G(5')ppp(5')G, 80 units of T7 RNA polymerase, and approximately 20,000 cpm of ^{32}P-CTP as a trace for quantitation of synthesized RNA by TCA precipitation. The reaction was incubated for 3 hrs. at 30°C; 20 units of RNase-free DNase was added, and the incubation was

allowed to proceed for an additional 15 min. at 37°C. cRNA was purified by two phenol, chloroform/isoamyl alcohol extractions, two ethanol precipitations, and resuspended at a concentration of 500 ng/ml in RNase-free water immediately before use.

EXAMPLE 5

Aequorin Bioluminescence Assay (ABA) and Clone Identification

The ABA requires injection of library pool cRNA (25 ng/egg for pool sizes of 500 to 10,000) with aequorin cRNA (2 ng/egg) supplemented with the G-protein alpha subunit Ga11 (2 ng/egg). To facilitate stabilization of synthetic transcripts from aequorin and Ga11 plasmids, the expression vector pCDNA-3 was modified (termed pcDNA-3v2) by insertion (in the Apa I restriction enzyme site of the polylinker) of a cassette to append a poly (A) tract on all cRNA's which initiate from the T7 RNA polymerase promoter. This cassette includes (5' to 3'): a Bgl II site, pA (20) and a Sfi I site which can be used for plasmid linearization. Polymerase chain reaction (PCR) was utilized to generate a DNA fragment corresponding to the open reading frame (ORF) of the aequorin cDNA with an optimized Kosak translational initiation sequence (Inouye, S. *et. al.*, 1985, *Proc. Natl. Acad. Sci. USA* 82:3154-3158). This DNA was ligated into pCDNA-3v2 linearized with EcoR I and Kpn I in the EcoR I/Kpn I site of pCDNA-3v2. Ga11 cDNA was excised as a Cla I/Not I fragment from the pCMV-5 vector (Woon, C. *et. al.*, 1989 *J. Biol. Chem.* 264: 5687-93), made blunt with Klenow DNA polymerase and inserted into the EcoR V site of pcDNA-3v2. cRNA was injected into oocytes using the motorized "Nanoject" injector (Drummond Sci. Co., Broomall, PA.) in a volume of 50 nl. Injection needles were pulled in a single step using a Flaming/Brown micropipette puller, Model P-87 (Sutter Instrument Co) and the tips were broken using 53X magnification such that an acute angle was generated with the outside diameter of the needle being <3 mm. Following injection, oocytes were incubated in ND-96 medium, with gentle orbital shaking at 18°C in the dark. Oocytes were incubated for 24 to 48 hours (depending on the experiment and the time required for expression of

the heterologous RNA) before "charging" the expressed aequorin with the essential chromophore coelenterazine. Oocytes were "charged" with coelenterazine by transferring them into 35 mm dishes containing 3 ml charging medium and incubating for 2-3 hours with gentle orbital shaking in the dark at 18°C. The charging medium contained 10 mM coelenterazine (Molecular Probes, Inc., Eugene, OR.) and 30 mM reduced glutathione in OR-2 media (no calcium). Oocytes were then returned to ND-86 medium with calcium medium described above and incubation continued in the dark with orbital shaking until bioluminescence measurements were initiated. Measurement of GHSR expression in oocytes was performed using a Berthold Luminometer LB953 (Wallac Inc., Gaithersburg, MD) connected to a PC running the Autolumat-PC Control software (Wallac Inc., Gaithersburg, MD). Oocytes (singly or in pairs) were transferred to plastic tubes (75 x 12 mm, Sarstedt) containing 2.9 ml Ca^{++}-free OR-2 medium. Each cRNA pool was tested using a minimum of 3 tubes containing oocytes. Bioluminescence measurements were triggered by the injection of 0.1 ml of 30 mM MK-677 (1 mM final concentration) and recordings were followed for 2 min. to observe kinetic responses consistent with an IP3-mediated response.

Pool S10-20 was prepared from the unfractionated swine pituitary cDNA library and was composed of 10 pools each of 1000 clones. S10-20 gave a positive signal on two luminometer instruments and the component pools were then individually tested for activity. From the 10 pools of 1000 clones, only pool S271 gave a positive response. This pool was made from two pools of 500 clones designated P541 and P542. Again, only one of the pools, P541, gave a positive bioluminescent signal in the presence of 1 mM Compound A. At this point, the bacterial titer was determined in the glycerol stock of P541 such that dilutions could be plated onto LB agar plates containing 100 mg/ml carbenicillin to yield approximately 50 colonies per plate. A total of 1527 colonies were picked and replicated from 34 plates. The colonies on the original plates were then washed off, plasmids isolated, cRNA synthesized and injected into oocytes. cRNA prepared from 8 of

the 34 plates gave positive signals in oocytes. Two plates were selected and the individual colonies from these plates were grown up, plasmid isolated, cRNA prepared and injected into oocytes. A single clonal isolate from each plate (designated as clones 7-3 and 28-18) gave a positive bioluminescence response to 1 mM Compound A. Clone 7-3 was further characterized.

EXAMPLE 6

Receptor Characterization

DNA sequencing was performed on both strands using an automated Applied Biosystems instrument (ABI model 373) and manually by the dideoxy chain termination method using Sequenase II (US Biochemical, Cleveland, OH). Database searches (Genbank 88, EMBL 42, Swiss-Prot 31, PIR 40, dEST, Prosite, dbGPCR), sequence alignments and analysis of the GHSR nucleotide and protein sequences were carried out using the GCG Sequence Analysis Software Package (Madison, WI; pileup, peptide structure and motif programs), FASTA and BLAST search programs, and the PC/Gene software suite from Intelligenetics (San Francisco, CA; protein analysis programs). Northern blot analysis was conducted using total (20 mg/lane) or poly (A)+ mRNA (5-10 mg/lane) prepared as described above. RNA was fractionated on a 1% agarose gel containing 2.2 M formaldehyde and blotted to a nitrocellulose membrane. Southern blots were hybridized with a PCR generated probe encompassing the majority of the ORF predicted by clone 7-3 (nt 291 to 1132). The probe was radiolabeled by random-priming with [a]^{32}P-dCTP to a specific activity of greater than 10^9 dpm/mg. Southern blots were pre-hybridized at 42°C for 4 hrs. in 5 X SSC, 5 x Denhardt's solution, 250 mg/ml tRNA, 1% glycine, 0.075% SDS, 50 mM $NaPO_4$ (pH 6) and 50% formamide. Hybridizations were carried out at 42°C for 20 hrs. in 5 X SSC, 1 X Denhardt's solution, 0.1% SDS, 50 mM $NaPO_4$, and 50% formamide. RNA blots were washed in 2 x SSC, 0.2% SDS at 42°C and at -70°C. RNA size markers were 28S and 18S rRNA and *in vitro* transcribed RNA markers (Novagen). Nylon membranes containing EcoR I and

Hind III digested genomic DNA from several species (Clontech; 10 mg/lane) were hybridized for 24 hrs. at 30°C in 6 X SSPE, 10 X Denhardt's, 1% SDS, and 50% formamide. Genomic blots were washed twice with room temperature 6 X SSPE, twice with 55°C 6 X SSPE, and twice with 55°C 4 X SSPE. Additional swine GHSR clones from the swine cDNA library (described above) were identified by hybridization to plasmid DNA (in pools of 500 clones each) immobilized to nylon membranes in a slot-blot apparatus (Scheicher and Schuell). Pure clonal isolates were subsequently identified by colony hybridization. Swine GHSR clones that extend further in a 5' direction were identified using 5' RACE procedures (Frohman, M. A., 1993 *Methods Enzymol.* 218:340-358, which is incorporated by reference) using swine pituitary poly $(A)^+$ mRNA as template.

EXAMPLE 7

Human GHSR

Human pituitary homologues of the swine GHSR were obtained by screening a commercially available cDNA library constructed in the vector lambda ZAP II (Stratagene) as per the manufacturer's instructions. Approximately 1.86×10^6 phages were initially plated and screened using a random-primer labeled portion of swine clone 7-3 (described above) as hybridization probe. Twenty one positive clones were plaque purified. The inserts from these clones were excised from the bacteriophage into the phagemid pBluescript II SK- by co-infection with helper phage as described by the manufacturer (Stratagene). Human clones were characterized as has been described above for the swine clone.

EXAMPLE 8

Assays

Mammalian cells (COS-7) were transfected with GHSR expression plasmids using Lipofectamine (GIBCO-BRL; Hawley-Nelson, P. 1993, *Focus* 15:73). Transfections were performed in 60 mm dishes on 80% confluent cells (approximately 4 x 10^5 cells) with 8 mg of Lipofectamine and 32 mg of GHSR plasmid DNA.

Binding of ^{35}S-Compound A to swine pituitary membranes and crude membranes prepared from COS-7 cells transfected with GHSR expression plasmids was conducted. Crude cell membranes from COS-7 transfectants were prepared on ice, 48 hrs. post-transfection. Each 60 mm dish was washed twice with 3 ml of PBS, once with 1 ml homogenization buffer (50 mM Tris-HCl [pH 7.4], 5 mM $MgCl_2$, 2.5 mM EDTA, 30 mg/ml bacitracin). 0.5 ml of homogenization buffer was added to each dish, cells were removed by scraping and then homogenized using a Polytron device (Brinkmann, Syosset, NY; 3 bursts of 10 sec. at setting 4). The homogenate was then centrifuged for 20 min. at 11,000 x g at 0°C and the resulting crude membrane pellet (chiefly containing cell membranes and nuclei) was resuspended in homogenization buffer supplemented with 0.06% BSA (0.1 ml/60 mm dish) and kept on ice. Binding reactions were performed at 20°C for 1 hr. in a total volume of 0.5 ml containing: 0.1 ml of membrane suspension, 10 ml of ^{35}S-Compound A (0.05 to 1 nM; specific activity approximately 900 Ci/mmol), 10 ml of competing drug and 380-390 ml of homogenization buffer. Bound radioligand was separated by rapid vacuum filtration (Brandel 48-well cell harvester) through GF/C filters pretreated for 1 hr. with 0.5% polyethylenimine. After application of the membrane suspension to the filter, the filters were washed 3 times with 3 ml each of ice cold 50 mM Tris-HCl [pH 7.4], 10 mM $MgCl_2$, 2.5 mM EDTA and 0.015% Triton X-100, and the bound radioactivity on the filers was quantitated by scintillation counting. Specific binding (> 90% of total) is defined as the difference between total binding and

non-specific binding conducted in the presence of 50 nM unlabeled Compound A.

EXAMPLE 9

Preparation of High Specific Activity Radioligand [^{35}S]-Compound A

[^{35}S]-Compound A was prepared from an appropriate precursor, N-[1(R)-[(1,2-dihydrospiro[3H-indole-3,4'-piperidin]-1'-yl)-carbonyl]-2-(phenyl-methyloxy)ethyl]-2-amino-t-butoxycarbonyl-2-methylpropan-amide, using methane [^{35}S]sulfonyl chloride as described in Dean DC, *et al.*, 1995, In: Allen J, Voges R (eds) Synthesis and Applications of Isotopically Labelled Compounds, John Wiley & Sons, New York, pp. 795-801. Purification by semi-preparative HPLC (Zorbax SB-phenyl column, 68% MeOH/water, 0.1% TFA, 5 ml/min) was followed by N-t-BOC cleavage using 15% trifluro-acetic acid in dichloromethane (25°C, 3 hr) to give [methylsulfonyl-^{35}S]Compound A in near quantitative yield. HPLC purification (Hamilton PRP-1 4.6x250 mm column, linear gradient of 50-75% methanol-water with 1 mM HCl over 30 min, 1.3 ml/min) provided the ligand in >99% radiochemical purity. The structure was established by HPLC coelution with unlabeled Compound A and by mass spectral analysis. The latter method also indicated a specific activity of ~1000 Ci/mmol.

EXAMPLE 10

DNA Encoding a Rat Growth Hormone Secretagogue Receptor (GHSR) Type Ia

Cross-hybridization under reduced stringency was the strategy utilized to isolate the rat GHSR type Ia. Approximately 10^6 phage plaques of a once-amplified rat pituitary cDNA library in lambda gt11 (RL1051b; Clontech, Palo Alto, CA) were plated on *E. coli* strain Y1090r$^-$. The plaques were transferred to maximum-strength Nytran (Schleicher & Schuell, Keene, NH) denatured, neutralized and screened with a 1.6 kb EcoRI/NotI fragment containing the entire coding and

untranslated regions of the swine GHSR, clone 7-3. The membranes were incubated at 30°C in prehybridization solution (50% formamide, 2 X Denhardts, 5 X SSPE, 0.1% SDS, 100 mg/ml salmon sperm DNA) for 3 hours followed by overnight incubation in hybridization solution (50% formamide, 2 X Denhardts, 5 X SSPE, 0.1% SDS, 10% dextran sulfate, 100 mg/ml salmon sperm DNA) with 1 x 10^6 cpm/ml of [^{32}P]-labeled probe. The probe was labeled with [^{32}P]dCTP using a random priming kit (Gibco BRL, Gaithersburg, ND). After hybridization the blots were washed two times each with 2 X SSC, 0.1% SDS (at 24°C, then 37°C, and finally 55°C). A single positive clone was isolated following three rounds of plaque purification. Phage containing the GHSR was eluted from plate plaques with 1x lambda buffer (0.1M NaCl, 0.01M $MgSO_4 \cdot 7H_2O$, 35mM Tris-HCl, pH 7.5) following overnight growth of approximately 200 pfu/150mm dish. After a ten minute centrifugation at 10,000 x g to remove debris, the phage solution was treated with 1 mg/ml RNAse A and DNAse I for thirty minutes at 24°C, followed by precipitation with 20% PEG (8000)/2M NaCl for two hours on ice, and collection by centrifugation at 10,000 x g for twenty minutes. Phage DNA was isolated by incubation in 0.1% SDS, 30mM EDTA, 50 mg/ml proteinase K for one hour at 68°C, with subsequent phenol (three times) and chloroform (twice) extraction before isopropanol precipitation overnight. The GHSR DNA insert (~6.4 kb) was sub-cloned from lambda gt11 into the plasmid vector Litmus 28 (New England Biolabs, Beverly, MA). 2 mg of phage DNA was heated to 65°C for ten minutes, then digested with 100 units BsiWI (New England Biolab, Bevely, MA) at 37°C overnight. A 6.5 kb fragment was gel purified, electroeluted and phenol/chloroform extracted prior to ligation to BsiWI-digested Litmus 28 vector.

Double-stranded DNA was sequenced on both strands on a ABI 373 automated sequencer using the ABI PRISM dye termination cycle sequencing ready reaction kit (Perkin Elmer; Foster City, CA).

Comparison of the complete ORF encoding the rat GHSR type Ia protein sequence to human and swine GHSR homologs reveals a

high degree of sequence identity (rat *vs.* human, 95.1 %; rat *vs.* swine 93.4 %).

For sequence comparisons and functional expression studies, a contiguous DNA fragment encoding the complete ORF (devoid of intervening sequence) for the rat GHSR type Ia was generated. The PCR was utilized to synthesize a amino-terminal fragment from Met-1 to Val-260 with EcoRI (5') and HpaI (3') restriction sites appended, while a carboxyl-terminal fragment was generated from Lys-261 to Thr-364 with Dra I (5') and Not I (3') restriction sites appended. The ORF construct was assembled into the mammalian expression vector pSV7 via a three-way ligation with EcoRI/Not I-digested pSV7, EcoRI/Hpa I-digested NH_2-terminal fragment, and Dra I/Not I-digested C-terminal fragment.

Functional activity of the ORF construct was assessed by transfecting (using lipofectamine; GIBCO/BRL) 5 mg of plasmid DNA into the aequorin expressing reporter cell line (293-AEQ17) cultured in 60 mm dishes. Following approximately 40 hours of expression the aequorin in the cells was charged for 2 hours with coelenterazine, the cells were harvested, washed and pelleted by low speed centrifugation into luminometer tubes. Functional activity was determined by measuring Compound A dependent mobilization of intracellular calcium and concomitant calcium induced aequorin bioluminescence. Shown in Fig. 26 are three replicate samples exhibiting Compound A-induced luminescent responses.

WHAT IS CLAIMED IS:

1. A receptor which is a member of the growth hormone family of receptors, free from receptor-associated proteins.

2. Growth hormone secretagogue receptor, free from receptor-associated proteins.

3. A growth hormone secretagogue receptor according to Claim 2 which is human.

4. A growth hormone secretagogue receptor according to Claim 2 which is from swine.

5. A growth hormone secretagogue receptor according to Claim 2 which is from rat.

6. Growth hormone secretagogue related receptor, free from receptor-associated proteins.

7. Isolated growth hormone secretagogue receptor.

8. A growth hormone secretagogue receptor according to Claim 7 which is human.

9. A growth hormone secretagogue receptor according to Claim 7 which is from swine.

10. A growth hormone secretagogue receptor according to Claim 7 which is from rat.

11. A receptor according to Claim 4 or 9 which comprises a full length receptor or which comprises the amino acid sequence as shown in any one of FIGURES 3 or 5.

12. A receptor according to Claim 3 or 8 which comprises the amino acid sequence as shown in any one of FIGURES 7, 8, 10 or 22.

13. A receptor according to Claim 5 or 10 which comprises the amino acid sequence shown in FIGURE 25.

14. A functional equivalent of a receptor of Claim 1.

15. A functional equivalent of a receptor of Claim 2.

16. A functional equivalent of a receptor of Claim 6.

17. A nucleic acid which encodes a receptor that is a member of the growth hormone secretagogue family of receptors, said nucleic acid being free from associated nucleic acids.

18. A nucleic acid which encodes a growth hormone secretagogue receptor or a functional equivalent, said nucleic acid being free from associated nucleic acids.

19. A nucleic acid according to Claim 18 which encodes human growth hormone secretagogue receptor, or a functional equivalent.

20. A nucleic acid according to Claim 18 which encodes swine growth hormone secretagogue receptor, or a functional equivalent.

21. A nucleic acid according to Claim 18 which encodes rat growth hormone secretagogue receptor, or a functional equivalent.

22. A nucleic acid according to Claim 17 which encodes a growth hormone secretagogue related to receptor.

23. A nucleic acid according to Claim 18 which is a DNA.

24. A nucleic acid according to Claim 23 which is shown in any one of FIGURES 1 or 4.

25. A nucleic acid according to Claim 23 which is shown in any one of FIGURES 6, 9 or 11.

26. A nucleic acid according to Claim 23 which is shown in any one of FIGURES 23 or 24.

27. A nucleic acid according to Claim 18 which is an RNA.

28. A vector comprising a nucleic acid which encodes a receptor which is a member of the growth hormone secretagogue family of receptors.

29. A vector comprising a nucleic acid which encodes a growth hormone secretagogue receptor, or a functional equivalent.

30. A vector according to Claim 29 which is selected from the group consisting of: plasmids, modified viruses, yeast artificial chromosomes, bacteriophages, cosmids and transposable elements.

31. A vector according to Claim 29 wherein the nucleic acid encodes human growth hormone secretagogue receptor or a functional equivalent.

32. A vector according to Claim 29 wherein the nucleic acid encodes swine growth hormone secretagogue receptor, or a functional equivalent.

33. A vector according to Claim 29 wherein the nucleic acid encodes rat growth hormone secretagogue receptor, or a functional equivalent.

34. A vector according to Claim 28 wherein the nucleic acid encodes a growth hormone secretagogue related receptor.

35. A host cell comprising a vector according to Claim 28.

36. A host cell comprising a vector according to Claim 28.

37. A host cell according to Claim 36 wherein the nucleic acid encodes human growth hormone secretagogue receptor, or a functional equivalent.

38. A host cell according to Claim 36 wherein the nucleic acid encodes swine growth hormone secretagogue receptor, or a functional equivalent.

39. A host cell according to Claim 36 wherein the nucleic acid encodes rat growth hormone secretagogue receptor, or a functional equivalent.

40. A nucleic acid encoding a GPCR clone that belongs to the GHSR family and that hybridizes with a nucleotide which encodes either human, swine or rat GHSR under reduced stringency of hybridization.

```
        10         20         30         40
         .          .          .          .
CCTCACGCTGCCAGACCTGGGCTGGGACGCTCCCCCTGAA   40
AACGACTCGCTAGTGGAGGAGCTGCTGCCGCTCTTCCCCA   80
CGCCGCTGTTGGCGGGCGTCACCGCCACCTGCGTGGCGCT  120
CTTCGTGGTGGGTATCGCGGGCAACCTGCTCACGATGCTG  160
GTAGTGTCACGCTTCCGCGAGATGCGCACCACCACCAACC  200

       210        220        230        240
         .          .          .          .
TCTACCTGTCCAGCATGGCCTTCTCCGACCTACTCATCTT  240
CCTCTGCATGCCCCTCGACCTCTTCCGCCTCTGGCAGTAC  280
CGGCCTTGGAACCTTGGCAACCTGCTCTGCAAACTCTTCC  320
AGTTCGTTAGCGAGAGCTGCACCTACGCCACAGTGCTCAC  360
CATCACCGCGCTGAGCGTCGAGCGCTACTTCGCCATCTGC  400

       410        420        430        440
         .          .          .          .
TTCCCGCTGCGGGCCAAGGTAGTGGTCACCAAGGGCCGGG  440
TAAAGCTGGTCATCCTGGTCATCTGGGCCGTGGCCTTCTG  480
CAGCGCCGGGCCCATCTTCGTGCTGGTCGGAGTGGAGCAT  520
GATAACGGCACTGACCCTCGGGACACCAACGAGTGCCGCG  560
CCACGGAGTTCGCCGTGCGCTCCGGGCTGCTTACCGTCAT  600

       610        620        630        640
         .          .          .          .
GGTCTGGGTGTCCAGTGTCTTCTTCTTCCTGCCTGTCTTC  640
TGCCTCACTGTGCTCTATAGCCTCATCGGCAGGAAGCTCT  680
GGCGGAGGAAGCGCGGCGAGGCGGCGGTGGGCTCCTCGCT  720
CAGGGACCAGAACCACAAACAAACCGTGAAAATGCTGGCT  760
GTAGTGGTGTTTGCTTTCATACTCTGCTGGCTGCCTTTCC  800

       810        820        830        840
         .          .          .          .
ATGTAGGGCGATATTTATTTTCCAAATCCTTGGAGCCTGG  840
CTCTGTGGAGATTGCTCAGATCAGCCAATACTGCAACCTC  880
GTGTCCTTTGTCCTCTTCTACCTCAGTGCGGCCATCAACC  920
CTATTCTGTACAACATCATGTCCAAGAAGTATCGGGTGGC  960
GGTGTTCAAACTGCTGGGATTTGAGCCCTTCTCACAGAGG 1000

      1010       1020       1030       1040
         .          .          .          .
AAACTCTCCACTCTGAAGGATGAAAGTTCTCGGGCCTGGA 1040
CAGAATCTAGTATTAATACATGA 1063
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FIG. 1

```
        10          20
         .           .
MLVVSRFREM RTTTNLYLSS   20
MAFSDLLIFL CMPLDLFRLW   40
QYRPWNLGNL LCKLFQFVSE   60
SCTYATVLTI TALSVERYFA   80
ICFPLRAKVV VTKGRVKLVI  100

       110         120
         .           .
LVIWAVAFCS AGPIFVLVGV  120
EHDNGTDPRD TNECRATEFA  140
VRSGLLTVMV WVSSVFFFLP  160
VFCLTVLYSL IGRKLWRRKR  180
GEAAVGSSLR DQNHKQTVKM  200

       210         220
         .           .
LAVVVFAFIL CWLPFHVGRY  220
LFSKSLEPGS VEIAQISQYC  240
NLVSFVLFYL SAAINPILYN  260
IMSKKYRVAV FKLLGFEPFS  280
QRKLSTLKDE SSRAWTESSI  300

       310         320
         .           .
NT 302
```

FIG.2

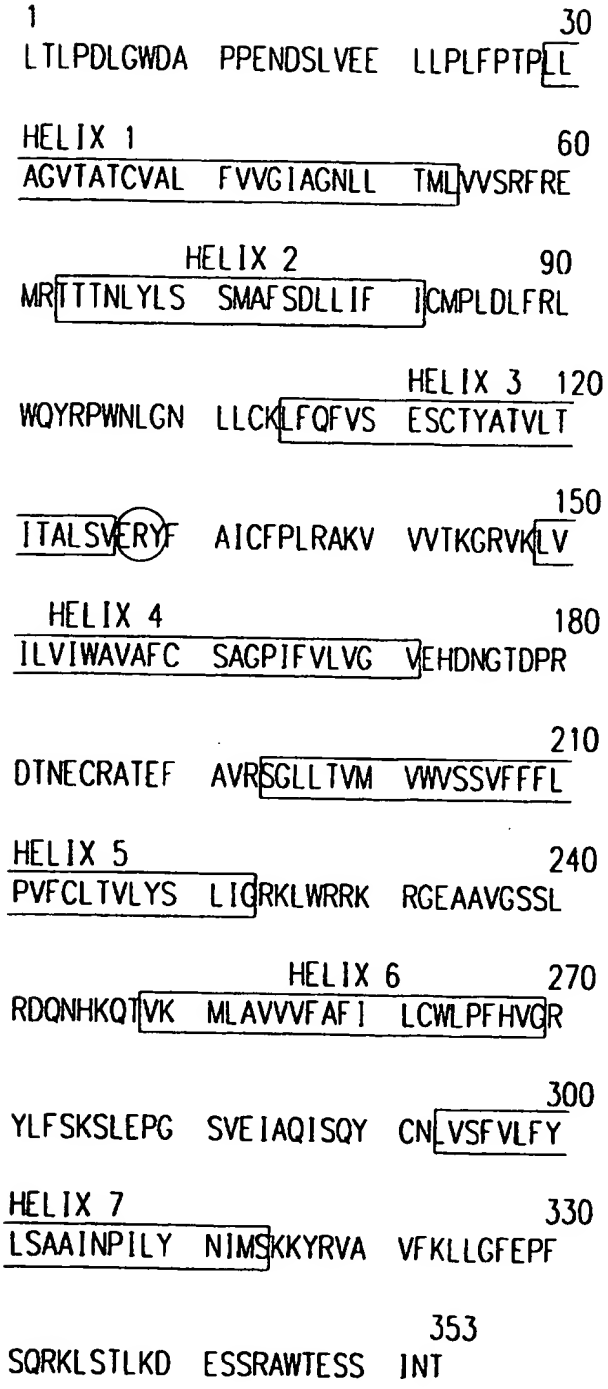

1
30
LTLPDLGWDA PPENDSLVEE LLPLFPTPLL
HELIX 1
60
AGVTATCVAL FVVGIAGNLL TMLVVSRFRE
HELIX 2
90
MRTTTNLYLS SMAFSDLLIF ICMPLDLFRL
HELIX 3
120
WQYRPWNLGN LLCKLFQFVS ESCTYATVLT
150
ITALSVERYF AICFPLRAKV VVTKGRVKLV
HELIX 4
180
ILVIWAVAFC SAGPIFVLVG VEHDNGTDPR
210
DTNECRATEF AVRSGLLTVM VWVSSVFFFL
HELIX 5
240
PVFCLTVLYS LIGRKLWRRK RGEAAVGSSL
HELIX 6
270
RDQNHKQTVK MLAVVVFAFI LCWLPFHVGR
300
YLFSKSLEPG SVEIAQISQY CNLVSFVLFY
HELIX 7
330
LSAAINPILY NIMSKKYRVA VFKLLGFEPF
353
SQRKLSTLKD ESSRAWTESS INT

FIG.3

```
        10         20         30         40
         .          .          .          .
GCAGCCTCTCACTTCCCTCTTTCCTCTCCTAGCATCCTCC    40
CTGAGAGCCCGCGCTCGATACTCCTTTGCACTCTTTCGCG    80
CCTAAGAGAACCTTCTCTGGGACCAGCCGGCTCCACCCTC   120
TCGGTCCTATCCAAGAGCCAGTTAAGCAGAGCCCTAAGCA   160
TGTGGAACGCGACCCCGAGCGAGGAACCGGGGCCCAACCT   200

       210        220        230        240
         .          .          .          .
CACGCTGCCAGACCTGGGCTGGGACGCTCCCCCTGAAAAC   240
GACTCGCTAGTGGAGGAGCTGCTGCCGCTCTTCCCCACGC   280
CGCTGTTGGCGGGCGTCACCGCCACCTGCGTGGCGCTCTT   320
CGTGGTGGGTATCGCGGGCAACCTGCTCACGATGCTGGTA   360
GTGTCACGCTTCCGCGAGATGCGCACCACCACCAACCTCT   400

       410        420        430        440
         .          .          .          .
ACCTGTCCAGCATGGCCTTCTCCGACCTACTCATCTTCCT   440
CTGCATGCCCCTCGACCTCTTCCGCCTTTGGCAGTACCGG   480
CCTTGGAACCTTGGCAACCTGCTCTGCAAACTCTTCCAGT   520
TCGTTAGCGAGAGCTGCACCTACGCCACAGTGCTCACCAT   560
CACCGCGCTGAGCGTCGAGCGCTACTTCGCCATCTGCTTC   600

       610        620        630        640
         .          .          .          .
CCGCTGCGGGCCAAGGTAGTGGTCACCAAGGGCCGGGTAA   640
AGCTGGTCATCCTGGTCATCTGGGCCGTGGCCTTCTGCAG   680
CGCCGGGCCCATCTTCGTGCTGGTCGGAGTGGAGCATGAT   720
AACGGCACTGACCCTCGGGACACCAACGAGTGCCGCGCCA   760
CGGAGTTCGCCGTGCGCTCCGGGCTGCTTACCGTCATGGT   800

       810        820        830        840
         .          .          .          .
CTGGGTGTCCAGTGTCTTCTTCTTCCTGCCTGTCTTCTGC   840
CTCACTGTGCTCTATAGCCTCATCGGCAGGAAGCTCTGGC   880
GGAGGAAGCGCGGCGAGGCGGCGGTGGGCTCCTCGCTCAG   920
GGACCAGAACCACAAACAAACCGTGAAAATGCTGGGTGGG   960
TCTCAATGCGCCCTCGAGCTTTCTCTCCCGGGTCCCCTCC  1000

      1010       1020       1030       1040
         .          .          .          .
ACTCCTCGTGCCTTTTCTCTTCTCCCTGA 1029
```

FIG.4

```
        10          20          30          40
         .           .           .           .
MWNATPSEEP GPNLTLPDLG WDAPPENDSL VEELLPLFPT   40
PLLAGVTATC VALFVVGIAG NLLTMLVVSR FREMRTTTNL   80
YLSSMAFSDL LIFLCMPLDL FRLWQYRPWN LGNLLCKLFQ  120
FVSESCTYAT VLTITALSVE RYFAICFPLR AKVVVTKGRV  160
KLVILVIWAV AFCSAGPIFV LVGVEHDNGT DPRDTNECRA  200

        210         220         230         240
         .           .           .           .
TEFAVRSGLL TVMVWVSSVF FFLPVFCLTV LYSLIGRKLW  240
RRKRGEAAVG SSLRDQNHKQ TVKMLGGSQC ALELSLPGPL  280
HSSCLFSSP  289
```

FIG.5

```
        10         20         30         40
         .          .          .          .
CGCCCAGCGAAGAGCCGGGGTTCAACCTCACACTGGCCGA    40
CCTGGACTGGGATGCTTCCCCCGGCAACGACTCGCTGGGC    80
GACGAGCTGCTGCAGCTCTTCCCCGCGCCGCTGCTGGCGG   120
GCGTCACAGCCACCTGCGTGGCACTCTTCGTGGTGGGTAT   160
CGCTGGCAACCTGCTCACCATGCTGGTGGTGTCGCGCTTC   200

       210        220        230        240
         .          .          .          .
CGCGAGCTGCGCACCACCACCAACCTCTACCTGTCCAGCA    240
TGGCCTTCTCCGATCTGCTCATCTTCCTCTGCATGCCCCT    280
GGACCTCGTTCGCCTCTGGCAGTACCGGCCCTGGAACTTC    320
GGCGACCTCCTCTGCAAACTCTTCCAATTCGTCAGTGAGA    360
GCTGCACCTACGCCACGGTGCTCACCATCACAGCGCTGAG    400

       410        420        430        440
         .          .          .          .
CGTCGAGCGCTACTTCGCCATCTGCTTCCCACTCCGGGCC    440
AAGGTGGTGGTCACCAAGGGGCGGGTGAAGCTGGTCATCT    480
TCGTCATCTGGGCCGTGGCCTTCTGCAGCGCCGGGCCCAT    520
CTTCGTGCTAGTCGGGGTGGAGCACGAGAACGGCACCGAC    560
CCTTGGGACACCAACGAGTGCCGCCCCACCGAGTTTGCGG    600

       610        620        630        640
         .          .          .          .
TGCGCTCTGGACTGCTCACGGTCATGGTGTGGGTGTCCAG    640
CATCTTCTTCTTCCTTCCTGTCTTCTGTCTCACGGTCCTC    680
TACAGTCTCATCGGCAGGAAGCTGTGGCGGAGGAGGCGCG    720
GCGATGCTGTCGTGGGTGCCTCGCTCAGGGACCAGAACCA    760
CAAGCAAACCGTGAAAATGCTGGCTGTAGTGGTGTTTGCC    800

       810        820        830        840
         .          .          .          .
TTCATCCTCTGCTGGCTCCCCTTCCACGTAGGGCGATATT    840
TATTTTCCAAATCCTTTGAGCCTGGCTCCTTGGAGATTGC    880
TCAGATCAGCCAGTACTGCAACCTCGTGTCCTTTGTCCTC    920
TTCTACCTCAGTGCTGCCATCAACCCCATTCTGTACAACA    960
TCATGTCCAAGAAGTACCGGGTGGCAGTGTTCAGACTTCT   1000

      1010       1020       1030       1040
         .          .          .          .
GGGATTCGAACCCTTCTCCCAGAGAAAGCTCTCCACTCTG   1040
AAAGATGAAAGTTCTCGGGCCTGGACAGAATCTAGTATTA   1080
ATACATGA  1088
```

FIG.6

```
          10                  20
           .                   .
MLVVSRFREL  RTTTNLYLSS   20
MAFSDLLIFL  CMPLDLVRLW   40
QYRPWNFGDL  LCKLFQFVSF   60
SCTYATVLTI  TALSVERYFA   80
ICFPLRAKVV  VTKGRVKLVI  100

         110                 120
           .                   .
FVIWAVAFCS  AGPIFVLVGV   120
EHENGTDPWD  TNECRPTEFA   140
VRSGLLTVMV  WVSSIFFFLP   160
VFCLTVLYSL  IGRKLWRRRR   180
GDAVVGASLR  DQNHKQTVKM   200

         210                 220
           .                   .
LAVVVFAFIL  CWLPFHVGRY   220
LFSKSFEPGS  LEIAQISQYC   240
NLVSFVLFYL  SAAINPILYN   260
IMSKKYRVAV  FRLLGFEPFS   280
QRKLSTLKDE  SSRAWTESSI   300

         310                 320
           .                   .
NT  302
```

FIG.7

1 30
PSEEPGFNLT LADLDWDASP GNDSLGDELL

HELIX 1 60
QLFPAPLLAG VTATCVALFV VGIAGNLLTM

HELIX 2 90
LVVSRFRELR TTTNLYLSSM AFSDLLIFLC

120
MPLDLVRLWQ YRPWNFGDLL CKLFQFVSES

HELIX 3 150
CTYATVLTIT ALSVERYFAI CFPLRAKVVV

HELIX 4 180
TKGRVKLVIF VIWAVAFCSA GPIFVLVGVE

210
HENGTDPWDT NECRPTEFAV RSGLLTVMVW

HELIX 5 240
VSSIFFFLPV FCLTVLYSLI GRKLWRRRRG

HELIX 6 270
DAVVGASLRD QNHKQTVKML AVVVFAFILC

300
WLPFHVGRYL FSKSFEPGSL EIAQISQYCN

HELIX 7 330
LVSFVLFYLS AAINPILYNI MSKKYRVAVF

360
RLLGFEPFSQ RKLSTLKDES SRAWTESSIN

361
T

FIG.8

```
        10         20         30         40
         .          .          .          .
GCGCCTCACGCTCCCGCTTCGCGGCGCCTGGTCCCTGCGG    40
TCCCCACTCGCTGCGACGCTTTGGGAAGTGCGAGATGGAA    80
CTGGATCGAGAACGCAAATGCGAGGCAGGGCTGGTGACAG   120
CATCCTCCCTACGCGTCTGCACCCGCTCCTCCCTCGCACC   160
CTCCCGCGCCTAAGCGGACCTCCTCGGGAGCCAGCTCGGT   200

        210        220        230        240
         .          .          .          .
CCAGCCTCCCAGCGCAGTCACGTCCCAGAGCCTGTTCAGC   240
TGAGCCGGCAGCATGTGGAACGCGACGCCCAGCGAAGAGC   280
CGGGGTTCAACCTCACACTGGCCGACCTGGACTGGGATGC   320
TTCCCCCGGCAACGACTCGCTGGGCGACGAGCTGCTGCAG   360
CTCTTCCCCGCGCCGCTGCTGGCGGGCGTCACAGCCACCT   400

        410        420        430        440
         .          .          .          .
GCGTGGCACTCTTCGTGGTGGGTATCGCTGGCAACCTGCT   440
CACCATGCTGGTGGTGTCGCGCTTCCGCGAGCTGCGCACC   480
ACCACCAACCTCTACCTGTCCAGCATGGCCTTCTCCGATC   520
TGCTCATCTTCCTCTGCATGCCCCTGGACCTCGTTCGCCT   560
CTGGCAGTACCGGCCCTGGAACTTCGGCGACCTCCTCTGC   600

        610        620        630        640
         .          .          .          .
AAACTCTTCCAATTCGTCAGTGAGAGCTGCACCTACGCCA   640
CGGTGCTCACCATCACAGCGCTGAGCGTCGAGCGCTACTT   680
CGCCATCTGCTTCCCACTCCGGGCCAAGGTGGTGGTCACC   720
AAGGGGCGGGTGAAGCTGGTCATCTTCGTCATCTGGGCCG   760
TGGCCTTCTGCAGCGCCGGGCCCATCTTCGTGCTAGTCGG   800

        810        820        830        840
         .          .          .          .
GGTGGAGCACGAGAACGGCACCGACCCTTGGGACACCAAC   840
GAGTGCCGCCCCACCGAGTTTGCGGTGCGCTCTGGACTGC   880
TCACGGTCATGGTGTGGGTGTCCAGCATCTTCTTCTTCCT   920
TCCTGTCTTCTGTCTCACGGTCCTCTACAGTCTCATCGGC   960
AGGAAGCTGTGGCGGAGGAGGCGCGGCGATGCTGTCGTGG  1000
```

FIG.9A

```
      1010        1020        1030        1040
       .           .           .           .
GTGCCTCGCTCAGGGACCAGAACCACAAGCAAACCGTGAA  1040
AATGCTGGGTGGGTCTCAGCGCGCGCTCAGGCTTTCTCTC  1080
GCGGGTCCTATCCTCTCCCTGTGCCTTCTCCCTTCTCTCI  1120
GA  1122
```

FIG.9B

```
        10         20         30         40
         .          .          .          .
MWNATPSEEPGFNLTLADLDWDASPGNDSLGDELLQLFPA   40
PLLAGVTATCVALFVVGIAGNLLTMLVVSRFRELRTTTNL   80
YLSSMAFSDLLIFLCMPLDLVRLWQYRPWNFGDLLCKLFQ  120
FVSESCTYATVLTITALSVERYFAICFPLRAKVVVTKGRV  160
KLVIFVIWAVAFCSAGPIFVIVGVEHENGTDPWDTNECRP  200

        210        220        230        240
         .          .          .          .
TEFAVRSGLLTVMVWVSSIFFFLPVFCLTVLYSLIGRKLW  240
RRRRGDAVVGASLRDQNHIKQTVKMLGGSQRALRLISIAGPI 280
LSLCLLPSL  289
```

FIG.10

```
        10         20         30         40
         .          .          .          .
MPLDLVRLWQYRPWNFGDLLCKLFQFVSESCTYATVLTIT   40
ALSVERYFAICFPLRAKVVVTKGRVKLVIFVIWAVAFCSA   80
GPIFVLVGVEHENGTDPWDTNECRPTEFAVRSGLLTVMVW  120
VSSIFFFLPVFCLTVLYSLIGRKLWRRRRGDAVVGASLRD  160
QNHKQTVKMLAVVVFAFILCWLPFHVGRYLFSKSFEPGSL  200

        210        220        230        240
         .          .          .          .
EIAQISQYCNLVSFVLFYLSAAINPILYNIMSKKYRVAVF  240
RLLGFEPFSQRKLSTLKDESSRAWTESSINT  271
```

FIG.12

```
        10         20         30         40
         .          .          .          .
ATCTGCTCATCTTCCTCTGCATGCCCCTGGACCTCGTTCG    40
CCTCTGGCAGTACCGGCCCTGGAACTTCGGCGACCTCCTC    80
TGCAAACTCTTCCAATTCGTCAGTGAGAGCTGCACCTACG   120
CCACGGTGCTCACCATCACAGCGCTGAGCGTCGAGCGCTA   160
CTTCGCCATCTGCTTCCCACTCCGGGCCAAGGTGGTGGTC   200

       210        220        230        240
         .          .          .          .
ACCAAGGGGCGGGTGAAGCTGGTCATCTTCGTCATCTGGG   240
CCGTGGCCTTCTGCAGCGCCGGGCCCATCTTCGTGCTAGT   280
CGGGGTGGAGCACGAGAACGGCACCGACCCTTGGGACACC   320
AACGAGTGCCGCCCCACCGAGTTTGCGGTGCGCTCTGGAC   360
TGCTCACGGTCATGGTGTGGGTGTCCAGCATCTTCTTCTT   400

       410        420        430        440
         .          .          .          .
CCTTCCTGTCTTCTGTCTCACGGTCCTCTACAGTCTCATC   440
GGCAGGAAGCTGTGGCGGAGGAGGCGCGGCGATGCTGTCG   480
TGGGTGCCTCGCTCAGGGACCAGAACCACAAGCAAACCGT   520
GAAAATGCTGGCTGTAGTGGTGTTTGCCTTCATCCTCTGC   560
TGGCTCCCCTTCCACGTAGGGCGATATTTATTTTCCAAAT   600

       610        620        630        640
         .          .          .          .
CCTTTGAGCCTGGCTCCTTGGAGATTGCTCAGATCAGCCA   640
GTACTGCAACCTCGTGTCCTTTGTCCTCTTCTACCTCAGT   680
GCTGCCATCAACCCCATTCTGTACAACATCATGTCCAAGA   720
AGTACCGGGTGGCAGTGTTCAGACTTCTGGGATTCGAACC   760
CTTCTCCCAGAGAAAGCTCTCCACTCTGAAAGATGAAAGT   800

       810        820        830        840
         .          .          .          .
TCTCGGGCCTGGACAGAATCTAGTATTAATACATGA   836
```

FIG.11

```
                                        v10         v20
FIG.3-SWINE TYPE I CLONE 7-3orf   LTLPDLGWDAPPENDSLVEE
                                  LTLPDLGWDAPPENDSLVEE
FIG.5-SWINE TYPE II CLONE 1375m   LTLPDLGWDAPPENDSLVEE
                                       ^20         ^30
                                          v30         v40
FIG.3-SWINE TYPE I CLONE 7-3orf   LLPLFPTPLLAGVTATCVAL
                                  LLPLFPTPLLAGVTATCVAI
FIG.5-SWINE TYPE II CLONE 1375m   LLPLFPTPLLAGVTATCVAI
                                       ^40         ^50
                                          v50         v60
FIG.3-SWINE TYPE I CLONE 7-3orf   FVVGIAGNLLTMLVVSRFRL
                                  FVVGIAGNLLTMLVVSRFRL
FIG.5-SWINE TYPE II CLONE 1375m   FVVGIAGNLLTMLVVSRFRL
                                       ^60         ^70
                                          v70         v80
FIG.3-SWINE TYPE I CLONE 7-3orf   MRTTTNLYLSSMAFSDLLIF
                                  MRTTTNLYLSSMAFSDLLIF
FIG.5-SWINE TYPE II CLONE 1375m   MRTTTNLYLSSMAFSDLLIF
                                       ^80         ^90
                                          v90         v100
FIG.3-SWINE TYPE I CLONE 7-3orf   LCMPLDLFRLWQYRPWNLGN
                                  LCMPLDLFRLWQYRPWNLGN
FIG.5-SWINE TYPE II CLONE 1375m   LCMPLDLFRLWQYRPWNLGN
                                       ^100        ^110
                                          v110        v120
FIG.3-SWINE TYPE I CLONE 7-3orf   LLCKLFQFVSESCTYATVLT
                                  LLCKLFQFVSESCTYATVLT
FIG.5-SWINE TYPE II CLONE 1375m   LLCKLFQFVSESCTYATVLT
                                       ^120        ^130
                                          v130        v140
FIG.3-SWINE TYPE I CLONE 7-3orf   ITALSVERYFAICFPLRAKV
                                  ITALSVERYFAICFPLRAKV
FIG.5-SWINE TYPE II CLONE 1375m   ITALSVERYFAICFPLRAKV
                                       ^140        ^150
                                          v150        v160
FIG.3-SWINE TYPE I CLONE 7-3orf   VVTKGRVKLVILVIWAVAFC
                                  VVTKGRVKLVILVIWAVAFC
FIG.5-SWINE TYPE II CLONE 1375m   VVTKGRVKLVILVIWAVAFC
                                       ^160        ^170
```

FIG.13A

```
                                        v170        v180
FIG.3-SWINE TYPE I CLONE 7-3orf     SAGPIFVLVGVEHDNGTDPR
                                    SAGPIFVLVGVFHDNGTDPR
FIG.5-SWINE TYPE II CLONE 1375m     SAGPIFVLVGVEHDNGIDPR
                                         ^180        ^190
                                        v190        v200
FIG.3-SWINE TYPE I CLONE 7-3orf     DTNECRATEFAVRSGLLTVM
                                    DTNECRATEFAVRSGLLTVM
FIG.5-SWINE TYPE II CLONE 1375m     DTNECRATEFAVRSGLLTVM
                                         ^200        ^210
                                        v210        v220
FIG.3-SWINE TYPE I CLONE 7-3orf     VWVSSVFFFLPVFCLTVLYS
                                    VWVSSVFFFLPVFCLTVLYS
FIG.5-SWINE TYPE II CLONE 1375m     VWVSSVFFFLPVFCLTVLYS
                                         ^220        ^230
                                        v230        v240
FIG.3-SWINE TYPE I CLONE 7-3orf     LIGRKLWRRKRGEAAVGSSL
                                    LIGRKLWRRKRGEAAVGSSL
FIG.5-SWINE TYPE II CLONE 1375m     LIGRKLWRRKRGEAAVGSSL
                                         ^240        ^250
                                        v250        v260
FIG.3-SWINE TYPE I CLONE 7-3orf     RDQNHKQTVKMLAVVVFAFI
                                    RDQNHKQTVKML:    A:
FIG.5-SWINE TYPE II CLONE 1375m     RDQNHKQTVKMLGGSQCALE
                                         ^260        ^270
                                        v270
FIG.3-SWINE TYPE I CLONE 7-3orf     LCWL-PFHVGRYLFSKS
                                    L.   P:H :..LFS.:
FIG.5-SWINE TYPE II CLONE 1375m     LSLPGPLH-SSCLFSSP
                                         ^280
```

FIG.13B

```
                                        v10         v20
FIG.8-HUMAN TYPE I 1146orf              PSEEPGFNLTLADLDWDASP
                                        PSEEPGFNLTLADLDWDASP
FIG.10-HUMAN TYPE II CLONE1141m         PSEEPGFNLTLADLDWDASP
                                            ^10         ^20
                                        v30         v40
FIG.8-HUMAN TYPE I 1146orf              GNDSLGDELLQLFPAPLLAG
                                        GNDSLGDELLQLFPAPLLAG
FIG.10-HUMAN TYPE II CLONE1141m         GNDSLGDELLQLFPAPLLAG
                                            ^30         ^40
                                        v50         v60
FIG.8-HUMAN TYPE I 1146orf              VTATCVALFVVGIAGNILTM
                                        VTATCVALFVVGIAGNILTM
FIG.10-HUMAN TYPE II CLONE1141m         VTATCVALFVVGIAGNLLTM
                                            ^50         ^60
                                        v70         v80
FIG.8-HUMAN TYPE I 1146orf              LVVSRFRELRTTTNLYLSSM
                                        LVVSRFRELRTTTNLYLSSM
FIG.10-HUMAN TYPE II CLONE1141m         LVVSRFRELRTTTNLYLSSM
                                            ^70         ^80
                                        v90         v100
FIG.8-HUMAN TYPE I 1146orf              AFSDLLIFLCMPLDLVRLWQ
                                        AFSDLLIFLCMPLDLVRLWQ
FIG.10-HUMAN TYPE II CLONE1141m         AFSDLLIFLCMPLDLVRLWQ
                                            ^90         ^100
                                        v110        v120
FIG.8-HUMAN TYPE I 1146orf              YRPWNFGDLLCKLFQFVSES
                                        YRPWNFGDLLCKLFQFVSES
FIG.10-HUMAN TYPE II CLONE1141m         YRPWNFGDLLCKLFQFVSES
                                            ^100        ^110
                                        v130        v140
FIG.8-HUMAN TYPE I 1146orf              CTYATVLTITALSVERYFAI
                                        CTYATVLTITALSVERYFAI
FIG.10-HUMAN TYPE II CLONE1141m         CTYATVLTITALSVERYFAI
                                            ^130        ^140

                                        v150        v160
FIG.8-HUMAN TYPE I 1146orf              CFPLRAKVVVTKGRVKLVIF
                                        CFPLRAKVVVTKGRVKLVIF
FIG.10-HUMAN TYPE II CLONE1141m         CFPLRAKVVVTKGRVKLVIF
                                            ^150        ^160
```

FIG.14A

```
                                        v170        v180
FIG.8-HUMAN TYPE I 1146orf              VIWAVAFCSAGPIFVLVGVF
                                        VIWAVAFCSAGPIFVLVGVF
FIG.10-HUMAN TYPE II CLONE1141m         VIWAVAFCSAGPIFVLVGVF
                                            ^170        ^180
                                                v190        v200
FIG.8-HUMAN TYPE I 1146orf              HENGTDPWDTNECRPTEFAV
                                        HENGTDPWDTNECRPTEFAV
FIG.10-HUMAN TYPE II CLONE1141m         HENGTDPWDTNECRPTEFAV
                                            ^190        ^200
                                                v210        v220
FIG.8-HUMAN TYPE I 1146orf              RSGLLTVMVWVSSIFFFLPV
                                        RSGLLTVMVWVSSIFFFLPV
FIG.10-HUMAN TYPE II CLONE1141m         RSGLLTVMVWVSSIFFFLPV
                                            ^210        ^220
                                                v230        v240
FIG.8-HUMAN TYPE I 1146orf              FCLTVLYSLIGRKLWRRRRG
                                        FCLTVLYSLIGRKLWRRRRG
FIG.10-HUMAN TYPE II CLONE1141m         FCLTVLYSLIGRKLWRRRRG
                                            ^230        ^240
                                                v250        v260
FIG.8-HUMAN TYPE I 1146orf              DAVVGASLRDQNHKQTVKML
                                        DAVVGASLRDQNHKQTVKML
FIG.10-HUMAN TYPE II CLONE1141m         DAVVGASLRDQNHKQTVKML
                                            ^250        ^260
```

FIG.14B

```
                                v10        v20        v30        v40
FIG.3-SWINE TYPE I CLONE 7-3orf LTLPDLGWDAPPENDSLVEELLPLFPTPLLAGVTATCVAL
                                LTL:DL:WDA:P.NDSL :ELL.LFP:PLLAGVTATCVAL
FIG.8-HUMAN TYPE I 1146orf      LTLADLDWDASPGNDSLGDELLQLFPAPLLAGVTATCVAL
                                 ^10        ^20        ^30        ^40
                                v50        v60        v70        v80
FIG.3-SWINE TYPE I CLONE 7-3orf FVVGIAGNLLTMLVVSRFREMRTTTNLYLSSMAFSDLLIF
                                FVVGIAGNLLTMLVVSRFRE:RTTTNLYLSSMAFSDLLIF
FIG.8-HUMAN TYPE I 1146orf      FVVGIAGNLITMLVVSRFRELRTTTNIYLSSMAFSDLLIF
                                 ^50        ^60        ^70        ^80
                                v90        v100       v110       v120
FIG.3-SWINE TYPE I CLONE 7-3orf LCMPLDLFRLWQYRPWNLGNLLCKLFQFVSESCTYATVLT
                                LCMPLDL RLWQYRPWN:G:LLCKLFQFVSESCTYATVLT
FIG.8-HUMAN TYPE I 1146orf      LCMPLDLVRLWQYRPWNFGDLLCKLFQFVSESCIYATVLI
                                 ^90        ^100       ^110       ^120
                                v130       v140       v150       v160
FIG.3-SWINE TYPE I CLONE 7-3orf ITALSVERYFAICFPLRAKVVVTKGRVKLVILVIWAVAFC
                                ITALSVERYFAICFPLRAKVVVTKGRVKLVI:VIWAVAFC
FIG.8-HUMAN TYPE I 1146orf      ITALSVERYFAICFPLRAKVVVTKGRVKLVIFVIWAVAFC
                                 ^130       ^140       ^150       ^160
                                v170       v180       v190       v200
FIG.3-SWINE TYPE I CLONE 7-3orf SAGPIFVLVGVEHDNGTDPRDTNECRATCFAVRSGLLTVM
                                SAGPIFVLVGVEH:NGTDP:DTNECR:TEFAVRSGLLTVM
FIG.8-HUMAN TYPE I 1146orf      SAGPIFVLVGVEHENGTDPWDTNECRPTEFAVRSGLLTVM
                                 ^170       ^180       ^190       ^200
                                v210       v220       v230       v240
FIG.3-SWINE TYPE I CLONE 7-3orf VWVSSVFFFLPVFCLTVLYSLIGRKLWRRKRGEAAVGSSL
                                VWVSS:FFFLPVFVLTVLYSLIGRKLWRR:RG:A.VG:SL
FIG.8-HUMAN TYPE I 1146orf      VWVSSIFFFLPVFCLTVLYSLIGRKLWRRRRGDAVVGASL
                                 ^210       ^220       ^230       ^240
                                v250       v260       v270       v280
FIG.3-SWINE TYPE I CLONE 7-3orf RDQNHKQTVKMLAVVVFAFILCWLPFHVGRYLFSKSLEPG
                                RDQNHKQTVKMLAVVVFAFILCWLPFHVGRYLFSKS:EPG
FIG.8-HUMAN TYPE I 1146orf      RDQNHKQTVKMLAVVVFAFILCWLPFHVGRYLFSKSFEPG
                                 ^250       ^260       ^270       ^280
                                v290       v300       v310       v320
FIG.3-SWINE TYPE I CLONE 7-3orf SVEIAQISQYCNLVSFVLFYLSAAINPILYNIMSKKYRVA
                                S:EIAQISQYCNLVSFVLFYLSAAINPILYNIMSKKYRVA
FIG.8-HUMAN TYPE I 1146orf      SLEIAQISQYCNLVSFVLIYLSAAINPILYNIMSKKYRVA
                                 ^290       ^300       ^310       ^320
                                v330       v340       v350
FIG.3-SWINE TYPE I CLONE 7-3orf VFKLLGFEPFSQRKLSTLKDESSRAWTESSINT
                                VF:LLGFEPFSQRKLSTLKDESSRAWTESSINT
FIG.8-HUMAN TYPE I 1146orf      VFRLLGFEPFSQRKLSTLKDESSRAWTESSINT
                                 ^330       ^340       ^350       ^360
```

FIG.15

```
                                    v10         v20
FIG.5-SWINE TYPE II CLONE 1375m     MWNATPSEEPGPNLTLPDLG
                                    MWNATPSEEPG NLTL:DL:
FIG.10-HUMAN TYPE II CLONE1141m     MWNATPSEEPGFNLTLADLD
                                        ^10         ^20
                                    v30         v40
FIG.5-SWINE TYPE II CLONE 1375m     WDAPPENDSLVEELLPIFPT
                                    WDA:P.NDSL :ELL.LFP:
FIG.10-HUMAN TYPE II CLONE1141m     WDASPGNDSLGDELLQLFPA
                                        ^30         ^40
                                    v50         v60
FIG.5-SWINE TYPE II CLONE 1375m     PLLAGVTATCVAIFVVGIAG
                                    PLLAGVTATCVAIFVVGIAG
FIG.10-HUMAN TYPE II CLONE1141m     PLLAGVTATCVALFVVGIAG
                                        ^50         ^60
                                    v70         v80
FIG.5-SWINE TYPE II CLONE 1375m     NLLTMLVVSRFREMRTTTNI
                                    NLLIMLVVSRFRE:RTTTNL
FIG.10-HUMAN TYPE II CLONE1141m     NLLTMLVVSRFRELRTTTNL
                                        ^70         ^80
                                    v90         v100
FIG.5-SWINE TYPE II CLONE 1375m     YLSSMAFSDLLIFLCMPLDL
                                    YLSSMAFSDLLIFLCMPLDL
FIG.10-HUMAN TYPE II CLONE1141m     YLSSMAFSDLLIFLCMPLDL
                                        ^90         ^100
                                    v110        v120
FIG.5-SWINE TYPE II CLONE 1375m     FRLWQYRPWNIGNLLCKLFQ
                                     RLWQYRPWN:G:LLCKLFQ
FIG.10-HUMAN TYPE II CLONE1141m     VRLWQYRPWNFGDLLCKLFQ
                                        ^110        ^120
                                    v130        v140
FIG.5-SWINE TYPE II CLONE 1375m     FVSESCTYATVLTITALSVE
                                    FVSESCTYATVLTITALSVE
FIG.10-HUMAN TYPE II CLONE1141m     FVSESCTYATVLTITALSVE
                                        ^130        ^140
                                    v150        v160
FIG.5-SWINE TYPE II CLONE 1375m     RYFAICFPLRAKVVVTKGRV
                                    RYFAICFPLRAKVVVTKGRV
FIG.10-HUMAN TYPE II CLONE1141m     RYFAICFPLRAKVVVTKGRV
                                        ^150        ^160
```

FIG.16A

```
                                        v170        v180
FIG.5-SWINE TYPE II CLONE 1375m         KLVILVIWAVAFCSAGPIFV
                                        KLVI:VIWAVAFCSAGPIFV
FIG.10-HUMAN TYPE II CLONE1141m         KLVIFVIWAVAFCSAGPIFV
                                        ^170        ^180
                                        v190        v200
FIG.5-SWINE TYPE II CLONE 1375m         LVGVEHDNGTDPRDTNECRA
                                        LVGVEH:NGTDP:DTNECR:
FIG.10-HUMAN TYPE II CLONE1141m         LVGVEHENGTDPWDTNECRP
                                        ^190        ^200
                                        v210        v220
FIG.5-SWINE TYPE II CLONE 1375m         TEFAVRSGLLTVMVWVSSVF
                                        TEFAVRSGLLTVMVWVSS:F
FIG.10-HUMAN TYPE II CLONE1141m         TFFAVRSGLLTVMVWVSSIF
                                        ^210        ^220
                                        v230        v240
FIG.5-SWINE TYPE II CLONE 1375m         FFLPVFCLTVLYSLIGRKLW
                                        FFLPVFCLTVLYSLIGRKLW
FIG.10-HUMAN TYPE II CLONE1141m         FFLPVFCLTVLYSLIGRKLW
                                        ^230        ^240
                                        v250        v260
FIG.5-SWINE TYPE II CLONE 1375m         RRKRGEAAVGSSLRDQNHKQ
                                        RR:RG:A.VG:SLRDQNHKQ
FIG.10-HUMAN TYPE II CLONE1141m         RRRRGDAVVGASLRDQNHKQ
                                        ^250        ^260
                                        v270        v280
FIG.5-SWINE TYPE II CLONE 1375m         TVKMLGGSQCALELSLPGPL
                                        TVKMLGGSQ AL LSL:GP:
FIG.10-HUMAN TYPE II CLONE1141m         TVKMLGGSQRALRLSLAGPI
                                        ^270        ^280

FIG.5-SWINE TYPE II CLONE 1375m         HSSCLFSS
                                         S CL::S
FIG.10-HUMAN TYPE II CLONE1141m         I SI.CLLPS
```

FIG.16B

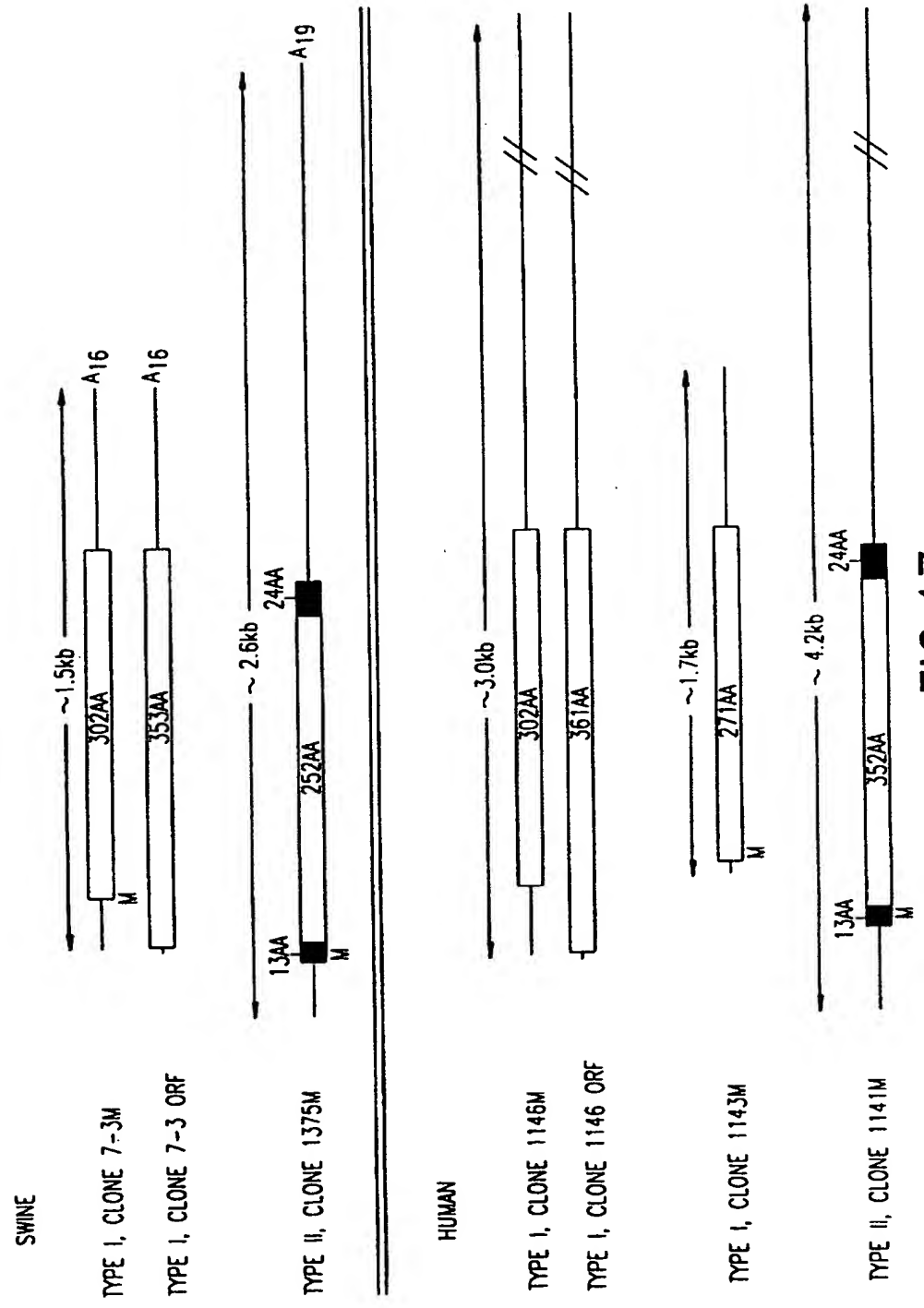

SWINE
TYPE I, CLONE 7-3M
~1.5kb
302AA
M
A16
TYPE I, CLONE 7-3 ORF
353AA
A16
TYPE II, CLONE 1375M
~2.6kb
24AA
13AA
252AA
M
A19
HUMAN
TYPE I, CLONE 1146M
~3.0kb
302AA
TYPE I, CLONE 1146 ORF
361AA
TYPE I, CLONE 1143M
~1.7kb
271AA
M
TYPE II, CLONE 1141M
~4.2kb
24AA
13AA
352AA
M

FIG. 17

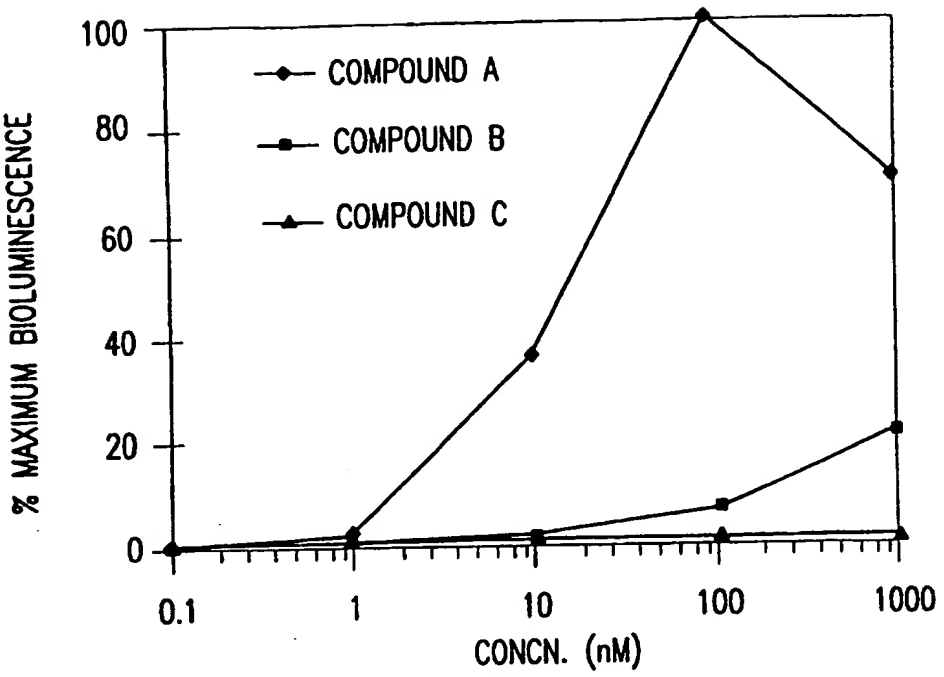


FIG.18

	SWINE CLONE 7-3		HUMAN CLONE 1146	
	24 HOURS	48 HOURS	24 HOURS	48 HOURS
COMPOUND A (100 μm)	13,553	2,692	1,353	2,228
(1000 nM)	9,176		3,091	
COMPOUND B (100nM)	717	425	113	108
COMPOUND C (100nM)	100	58	96	67
		3,839		
		1,806		
GHRP-2 (1000 nM)	2,492		1542	
GHRP-6 (1000 nM)	5,003		617	

FIG.19

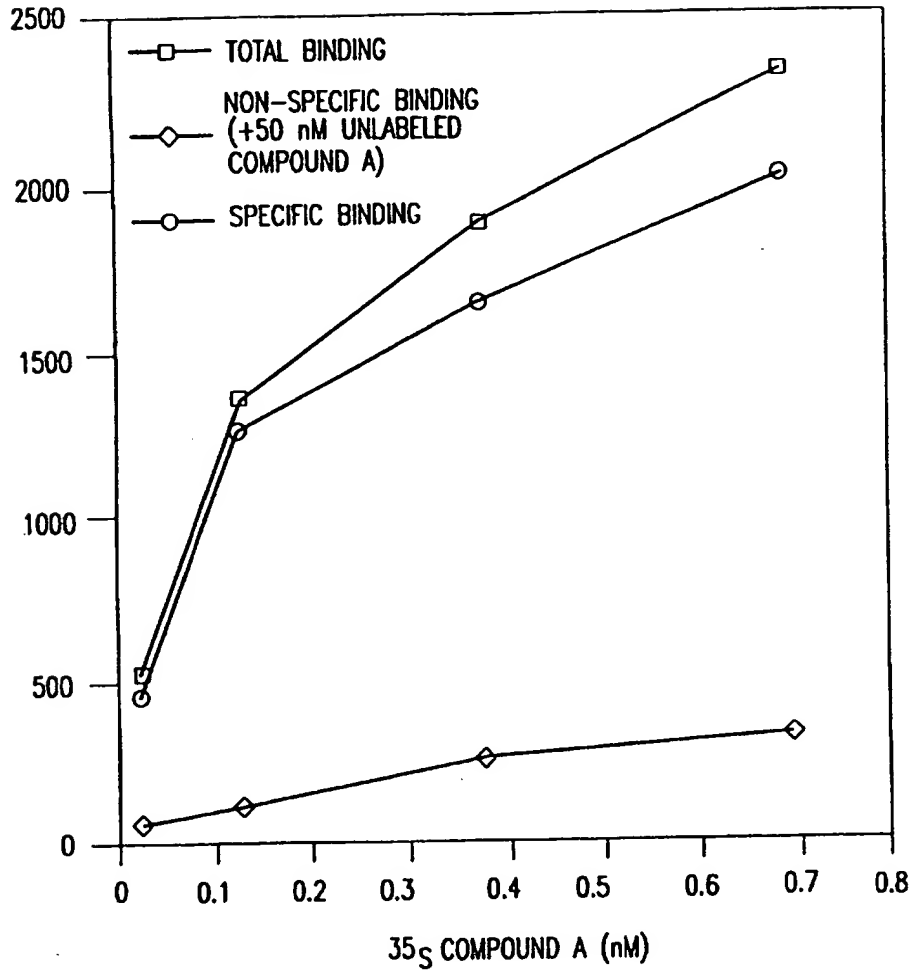


FIG.20

LIGAND	INHIBITION (% OF CONTROL SPECIFIC BINDING)
COMPOUND A @ 5nM	97
GHRP-6 @ 10nM	84
COMPOUND C @ 1 μM	43
GALAMIN @ 10 μM	44
AMENOMEDIN N @ 10 μM	19

FIG.21

```
  1  MWNATPSEEP GFNLTLADLD WDASPGNDSL GDELLQLFPA PLLAGVTATC
 51  VALFVVGIAG NLLTMLVVSR FRELRTTTNL YLSSMAFSDL LIFLCMPLDL
101  VRLWQYRPWN FGDLLCKLFQ FVSESCTYAT VLTITALSVE RYFAICFPLR
151  AKVVVTKGRV KLVIFVIWAV AFCSAGPIFV LVGVEHENGT DPWDTNECRP
201  TEFAVRSGLL TVMVWVSSIF FFLPVFCLTV LYSLIGRKLW RRRRGDAVVG
251  ASLRDQNHKQ TVKMLAVVVF AFILCWLPFH VGRYLFSKSF EPGSLEIAQI
301  SQYCNLVSFV LFYLSAAINP ILYNIMSKKY RVAVFRLLGF EPFSQRKLST
351  LKDESSRAWT ESSINT*
```

FIG.22

```
        10        20        30        40        50        60
ATG TGG AAC GCG ACC CCC AGC GAG GAG CCG GAG CCT AAC GTC ACG TTG GAC CTG GAT TGG   60
GAC GCT TCC CCC GGC AAC GAC TCA CTG CCT GAC GAA CTG CTG CCG CTG TTC CCC GCT CCG  120
CTG CTG GCA GGC GTC ACC GCC ACC TGC GTG GCG CTC TTC GTG GTG GGC ATC TCA GGC AAC  180
CTG CTC ACT ATG CTG GTG GTG TCC CGC TTC CGC GAG CTG CGC ACC ACC ACC AAC CTC TAC  240
CTG TCC AGC ATG GCC TTC TCG GAT CTG CTC ATC TTC CTG TGC ATG CCG CTG GAC CTC GTC  300

        310       320       330       340       350       360
CGC CTC TGG CAG TAC CGG CCC TGG AAC TTC GGC GAC CTG CTC TGC AAA CTC TTC CAG TTT  360
GTC AGC GAG AGC TGC ACC TAC GCC ACG GTC CTC ACC ATC ACC GCG CTG AGC GTC GAG CGC  420
TAC TTC GCC ATC TGC TTC CCT CTG CGG GCC AAG GTG GTG GTC ACT AAG GGC CGC GTG AAG  480
CTG GTC ATC CTT GTC ATC TGG GCC GTG GCT TTC TGC AGC GCG GGG CCC ATC TTC GTG CTG  540
GTG GGC GTG GAG CAC GAA AAC GGC ACA GAT CCC CGG GAC ACC AAC GAA TGC CGC GCC ACC  600

        610       620       630       640       650       660
GAG TTC GCT GTG CGC TCT GGG CTG CTC ACC GTC ATG GTG TGG GTG TCC AGC GTC TTC TTC  660
TTT CTA CCG GTC TTC TGC CTC ACT GTG CTC TAC AGT CTC ATC GGG AGG AAG CTA TCG CGG  720
AGA CGC GGA GAT GCA GCG GTG GGC GCC TCG CTC CGG GAC CAG AAC CAC AAG CAG ACA GTG  780
AAG ATG CTT Ggt gag tcc tgg cac ccg ctg acc ttt ctt ccc cca ctg cct gcc ctt ccc  840
cag cgg cct cta ttt ctg ttt ctc atc atc tcc gct gct ccc caa gtc tct caa gtc tct gtc  900
```

FIG.23A

```
        910         920         930         940         950         960
         .           .           .           .           .           .
ttt ctc tgc ctc tct cac ctt ggt tct cgg tct cac tgc ttt ctg ttt tct tcc tgt ctt   960
ttc ctg tat ctt gtc cac gaa aaa gaa ccc tca tat tgg taa ttc ctt aaa acg agg aac  1020
ctt ggt ctg gga aaa ttg gtc caa gat gga aat acc tca cgg ttt att gag ccc cta att  1080
gtt aac ggt tta gct tct tgt ctc aca tag aat ttg tgg tta tca aag taa taa tat taa  1140
ggt aag cag gca ggt aat ggg ttt aga aat cac tcc atg gta agt cta acc aca aat ttg  1200

        1210        1220        1230        1240        1250        1260
         .           .           .           .           .           .
ggt cac tct gtt aag gac ggc tta tag atg tat ttt gtt tgt ttt caa tat tgg gat ttg  1260
ttt tct gcc ctg cat ctt tct cag ata att aca tcc act ctg ttt agt cta tgg ttt tgc  1320
cag gag ggg ctt cat gct ggg gtc tcc ttt ttc ttg ttt ttg tat ttg tct ccc cag taa  1380
tat agg cca gga tag ggt gga gaa gtc atc ctt tcc tca aac tgt cct tca gga agg tct  1440
ggg tac tga acg gtt act gca taa act ctg ctt ccc caa agg cat gtg ctt ggt gtg gta  1500

        1510        1520        1530        1540        1550        1560
         .           .           .           .           .           .
aag tca tga aga tgg tgc tca tgt cca aga gga acc tct gat ctc act ttt caa ggg att  1560
tca tgt ttg ctg aca ttt aat act tgt tag ttt ttg cag ggg gat gat ttc tca ttt gca  1620
att tta tta ttc tca aat tct gca tgt cag aat gtt aga gat ttc tca ggg atg tca ggt  1680
tct gtt tcc aga tga gtg att gcc ctg tgt cct cca ttg gac tgt aaa ctc ata tgc acc  1740
aga cag ggt cta cat tgc tgc cgt ggt gca tag cct tcc atg tgt cac tta gtc cta aag  1800
```

FIG.23B

```
        1810        1820        1830        1840        1850        1860
aga agt tac taa taa cct aat ctc act aat ctc act ggc atc tca atg ccg atc cca ttg  1860
tca tct gaa aat ttg aag ggg aca tta aag tgg cac agg gac cag aac aat att ttt ctc  1920
tca ttg ctg aat ttt aaa aac aat cta aaa aat tgg aat tct tga aga aac tat ctt ata  1980
tga cta aaa tga agc ctt ggg tgg gtg cta att att att gtc tgg ctt acc tgc ccc ccc  2040
cac tac tta tat ctt tta gag atg aca cag act tgc ttt ccc tgt ggc tac taa tcc caa  2100

        2110        2120        2130        2140        2150        2160
ttg cac att cag tcc ctt gat aga ctt act cta aaa atc taa gtt cag cgg tcc acg aaa  2160
cat aac aaa gcc tgt cct aaa aca gca aga aag aaa gaa aga aag aaa gca aga aag aaa  2220
gca aga aag aaa gaa aga aaa cag aag aca aac aag gtc ttt ccc cat tcc cta aca tac  2280
agg aat gga aat tat taa gtc tac gtg ata gcc aat gca tct gtt tct tca gta tgc cca  2340
caa ggg tgc tgc cgg agc cat tgc tca ggg ctg gag tat tta ctg ggc atg ctt gac ccc  2400

        2410        2420        2430        2440        2450        2460
agc atg gag ggt gag aag tgc tcc tgg gaa ctc tga tcc act gct gtg gtg gag agc aaa  2460
cac ctg gcc tca ttt ata ctt gtt gtc tgt ata atg cat ata aat ggg gga taa tca tta  2520
cta aac tgt tta gct gag cct cat gtc agt caa tca caa agc aga gta att acc aca cag  2580
act ggg aag ctc agt gaa gat tgt tag cgg ttg gtc tga cag tct tgc tgt gtg cta tag  2640
tgt tag acc caa cgg agg cag tat tta toa gga ggg cag ggt tcc atg ttt ccc gtg tta  2700
```

FIG.23C

```
2710        2720        2730        2740        2750        2760

aag agc aag aga tga tgt ttg tca gta ggc atg cag ctc atg gtg aaa aga aag tcc aga  2760
ctt aaa gat gtg aag tga ttt gtg ctt tgc ccc acc ctg aca gtc tct ctc tgt gtg cst  2820
tca GCT GTG GTG GTG TTT GCT TTC ATC CTC TGC TGG CTG CCC TTC CAC GTG GGA AGA TAC  2880
CTC TTT TCC AAG TCC TTC GAG CCT GGC TCT CTG GAG ATC GCT CAG ATC AGC CAG TAC TGC  2940
AAC CTG GTG TCC TTT GTC CTC TTC TAC CTC AGC GCT GCC ATC AAC CCC ATT CTG TAC AAC  3000

      3010        3020        3030        3040        3050        3060

ATC ATG TCC AAG AAG TAC CGG GTG GCA GTG TTC AAA CTG CTA GGA TTT GAA TCC TTC TCC  3060
CAG AGA AAG CTT TCC ACT CTG AAG GAT GAG AGT TCC CGG GCC TGG ACA AAG TCG AGC ATC  3120
AAC ACA TGA  3129
```

FIG.23D

```
        10        20        30        40        50        60
ATG TGG AAC GCG ACC CCC AGC GAG GAG CCG GAG CCT AAC GTC ACG TTG GAC CTG GAT TGG   60
GAC GCT TCC CCC GGC AAC GAC TCA CTG CCT GAC GAA CTG CTG CCG CTG TTC CCC GCT CCG  120
CTG CTG GCA GGC GTC ACC GCC ACC TGC GTG GCG CTC TTC GTG GTG GGC ATC TCA GGC AAC  180
CTG CTC ACT ATG CTG GTG GTG TCC CGC TTC CGC GAG CTG CGC ACC ACC ACC AAC CTC TAC  240
CTG TCC AGC ATG GCC TTC TCG GAT CTG CTC ATC TTC CTG TGC ATG CCG CTG GAC CTC GTC  300

      310       320       330       340       350       360
CGC CTC TGG CAG TAC CGG CCC TGG AAC TTC GGC GAC CTG CTC TGC AAA CTC TTC CAG TTT  360
GTC ACC GAG AGC TGC ACC TAC GCC ACG GTC CTC ACC ATC ACC GCG CTG AGC GTC GAG CGC  420
TAC TTC GCC ATC TGC TTC CCT CTG CGG GCC AAG GTG GTG GTC ACT AAG GGC CGC GTG AAG  480
CTG GTC ATC CTT GTC ATC TGG GCC GTG GCT TTC TGC AGC GCG GGG CCC ATC TTC GTG CTG  540
GTG GGC GTG GAG CAC GAA AAC GGC ACA GAT CCC CGG GAC ACC AAC GAA TGC CGC GCC ACC  600

      610       620       630       640       650       660
GAG TTC GCT GTG CGC TCT GGG CTG CTC ACC GTC ATG GTG TGG GTG TCC AGC GTC TTC TTC  660
TTT CTA CCG GTC TTC TGC CTC ACT GTG CTC TAC AGT CTC ATC GGG AGG AAG CTA TGG CGG  720
AGA CGC GGA GAT GCA GCG GTG GGC GCC TCG CTC CGG GAC CAG AAC CAC AAG CAG ACA GTG  780
AAG ATG CTT GCT GTG GTG TTT GCT TTC ATC CTC TGC TGG CTG CCC TTC CAC GTG GGA  840
AGA TAC CTC TTT TCC AAG TCC TTC GAG CCT GGC TCT CTG GAG ATC GCT CAG ATC AGC CAG  900
```

FIG.24A

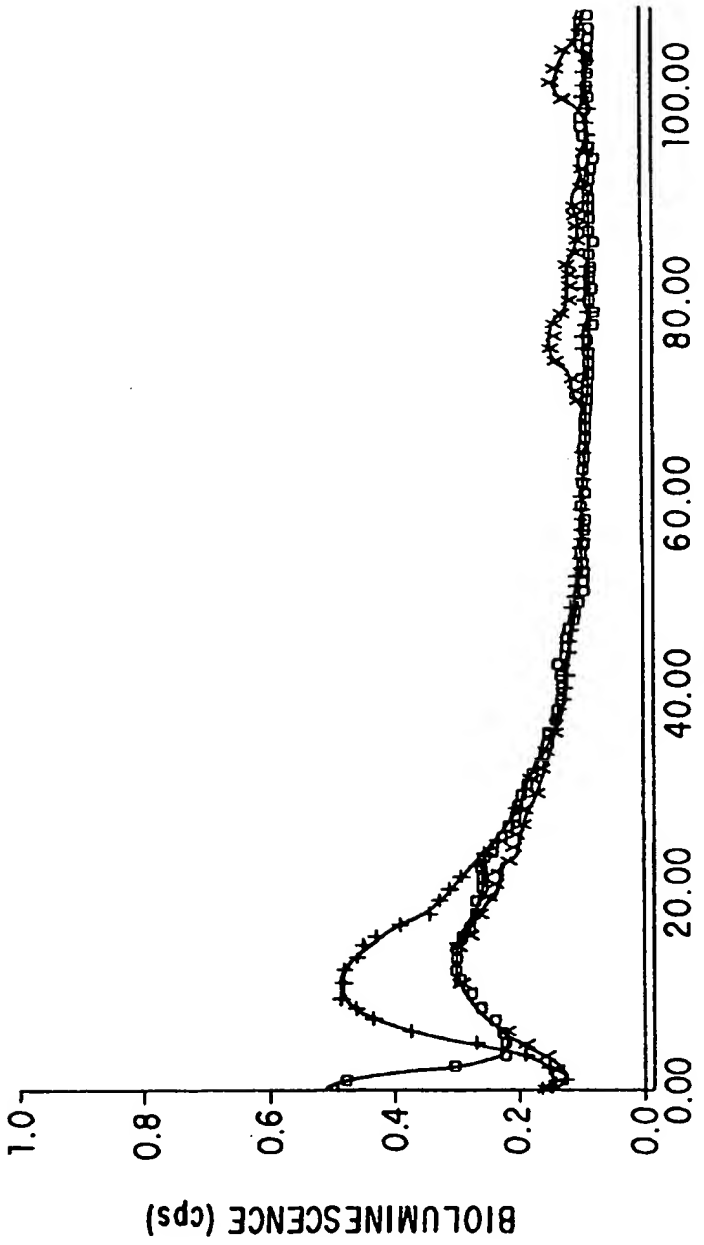
```
                910       920       930       940       950       960
TAC TGC AAC CTG GTG TCC TTT GTC CTC TTC TAC CTC AGC GCT GCC ATC AAC CCC ATT CTG   960
TAC AAC ATC ATG TCC AAG AAG TAC CGG GTG GCA GTG TTC AAA CTG CTA GGA TTT GAA TCC  1020
TTC TCC CAG AGA AAG CTT TCC ACT CTG AAG GAT GAG AGT TCC CGG GCC TGG ACA AAG TCG  1080
AGC ATC AAC ACA  1092
```

FIG.24B

```
        10         20         30         40         50
         .          .          .          .          .
MWNATPSEEP EPNVTLDLDW DASPGNDSLP DELLPLFPAP LLAGVTATCV    50
ALFVVGISGN LLTNLVVSRF RELRTTTNLY LSSMAFSDLL IFLCMPLDLV   100
RLWQYRPWNF GDLLCKLFQF VSESCTYATV LTITALSVER YFAICFPLRA   150
KVVVTKGRVK LVILVIWAVA FCSAGPIFVL VGVEHENGTD PRDTNECRAT   200
EFAVRSGLLT VMVWVSSVFF FLPVFCLTVL YSLIGRKLWR RRGDAAVGAS   250

       260        270        280        290        300
         .          .          .          .          .
LRDQNHKQTV KMLAVVVFAF ILCWLPFHVG RYLFSKSFEP GSLEIAQISQ   300
YCNLVSFVLF YLSAAINPIL YNIMSKKYRV AVFKLLGFES FSQRKLSTLK   350
DESSRAWTKS SINT  364
```

FIG.25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1.0
0.8
0.6
0.4
0.2
0.0
0.00
20.00
40.00
60.00
80.00
100.00
TIME (SEC)

FIG. 26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19445

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 300; 536/23.1, 23.5; 435/69.1, 70.1, 320.1, 336, 365, 252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, CAPLUS, WPIDS
search terms: growth hormone, receptor, secretagogue, human, rat, swine, sequence, DNA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,057,417 (HAMMONDS ET AL) 15 October 1991 , column 3, lines 24-39.	1
Y, E	US 5,583,010 (BAUMBACH ET AL) 10 December 1996 , entire document.	1, 2, 4, 6, 7, 9, 14-18, 20, 22, 23, 28-30, 32, 34-36, 38, 40
X, P ------ Y, P	HOWARD ET AL. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science. 16 August 1996, Vol.273, pages 974-977, see entire document.	17-20, 22-23, 27-32, 34-38,40 ---------- 1-10,14-16, 21, 33, 39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 FEBRUARY 1997	Date of mailing of the international search report 14 APR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ELIANE LAZAR-WESLEY Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19445

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ALOI ET AL. Neuroendocrine responses to a novel growth hormone secretagogue, L-692,429, in healthy older subjects. Journal of Clinical Endocrinology and Metabolism. October 1994, Vol.79, No.4, pages 943-949, especially last paragraph.	1-10,14-23,27-40
A	BOWERS, C.Y. Editorial: On a peptidomimetic growth hormone-releasing peptide.Journal of Clinical Endocrinology and Metabolism. October 1994. Vol.79, No.4, pages 940-942.	1-10, 14-23, 27-40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19445

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 11-13 and 24-26
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19445

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07K 14/705, 14/72, 14/435, 14/60, 14/61, 14/47; C12N 15/12, 15/09, 15/10, 15/00, 5/10

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/350, 300; 536/23.1, 23.5; 435/69.1, 70.1, 320.1, 336, 365, 252.3

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

Because a computer-readable copy of the sequence listing was not available, claims 11-13 and 24-26 were unsearchable to the extent that no meaningful search of the sequences per se can be carried out by this Authority. However, the subject matter of the claims has been searched to the extent possible with reference to to the balance of the description.